Vol. XX September, 1943 No. 5

CEREAL CHEMISTRY

Published bi-monthly by the American Association of Cereal Chemists
at Prince and Lemon Sts., Lancaster, Pa.

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* With the exception of the 1942–43 Committee Reports all papers in this issue were prepared for publication by the former editorial staff.

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from, and subscriptions placed with the Managing Editor, Prince and Lemon Sts., Lancaster, Pa., or Agricultural Experiment Station, Lincoln, Nebraska. Subscription rates, $6 per year. Foreign postage, 50 cents extra. Single copies, $1.25; foreign, $1.35.

Entered as second-class matter March 3, 1932, at the post office at Lancaster, Pa., under the act of August 24, 1912.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized February 16, 1924.

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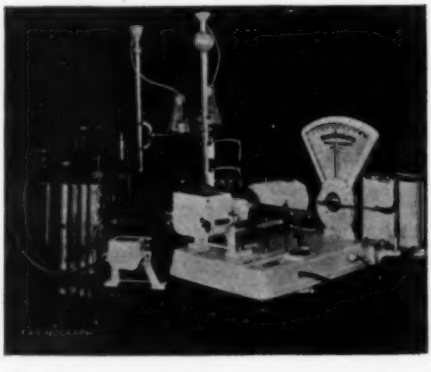

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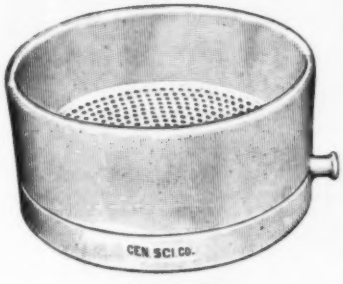

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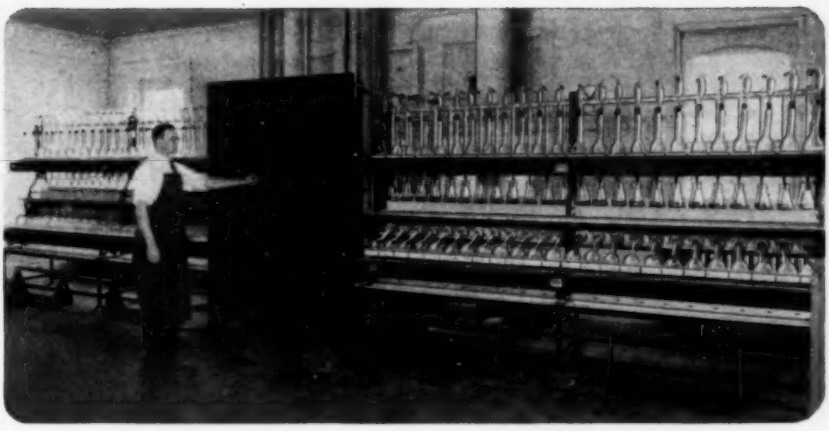

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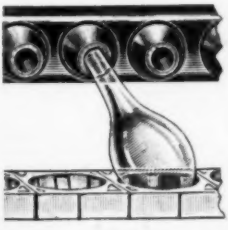

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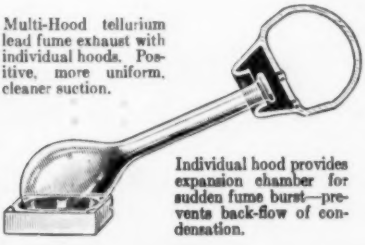

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CEREAL CHEMISTRY

VOL. XX SEPTEMBER, 1943 No. 5

THE RELATION OF PROTEOLYSIS TO THE CHARACTERISTICS OF OXIDATION AND REDUCTION IN DOUGHS[1]

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Department of Agricultural Chemistry, Nebraska Agricultural Experiment Station, Lincoln, Nebraska

(Read at the Annual Meeting, May 1942)

The role of proteolysis in bread doughs has been the subject of considerable investigation and controversy. The suggestion was made by Jørgensen (1935) and Balls and Hale (1936a) that the action of oxidizing agents in bread doughs can be explained on the basis of the inhibition of proteinase action. According to this theory such characteristics of unoxidized doughs as the pliability, extensibility, poor gas retention, and lack of oven spring (generally referred to as "young" or "green" doughs) and the small underdeveloped loaf of bread with its round, thick-walled cells ("young" or "underdeveloped") are the results of proteinase action. The intensification of these characteristics (production of soft, sticky doughs) on the addition of certain reducing agents is explained as being due to the activation of the proteinases or (Balls and Hale, 1936b) to an increased susceptibility of the gluten to proteolytic attack. The characteristics of oxidation ("age" or "overdevelopment") in doughs are explained as being the result of the inhibition of the proteinases, with a corresponding absence of proteolysis. The theory is based on the similarity in the properties produced in doughs and in bread by added proteinases and on the fact that proteinases of the papain type can be activated by certain reducing agents and inhibited by certain oxidizing agents. The proteinases of wheat flour have been said to be of the papain type (Balls and Hale, 1935 and 1938).

It has been pointed out by a number of workers that the theory of the activation of proteinases, as the explanation of the action of reducing agents, does not correspond with the observed action of these

[1] Published with the approval of the Director as paper No. 335. Journal Series, Nebraska Agricultural Experiment Station.

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agents in doughs. Ford and Maiden (1938), in comparing the effects of glutathione and papain in doughs, showed that glutathione had a direct and immediate softening effect on the flour protein and that the effect did not increase with time (as it would if it were acting as a proteinase activator); while papain had a delayed softening effect which increased with time of action. Balls and Hale (1936b) themselves show that the action of reducing agents is rapid and complete in a short period and that this does not indicate proteolytic activation but direct action on the gluten. They then suggest that the protein is activated and thus made more susceptible to proteinase action. However, this assumption of an activated protein still includes the theory that the reduced character of the dough is due to proteinase action (on the activated gluten) and accordingly would be little more compatible with observed rates of action than the original theory.

Elion (1943) in his defense of the proteolysis theory appears to consider the active proteolytic enzyme only as a detrimental ingredient, the detrimental effect of the enzyme being due merely to its presence, not to the degradation which it produces in the gluten. His discussion seems to imply that on inactivation of the proteinase the gluten should also regain its original properties—that is, ". . . a progressive return of the flour proteinases to their latent or inactive state and a progressive decrease of proteolytic activity in the dough . . . with a progressive improvement of its quality." It seems to us that this view of proteolysis and its inhibition is untenable.

The literature, pro and con, on this topic and on the general topic of oxidation and reduction in doughs has been thoroughly reviewed by Sullivan, Howe, Schmalz, and Astleford (1940) and by Shen and Geddes (1942). These reviews point out the exceeding complexity of the question of the probable action of oxidizing and reducing agents in doughs and the inadequacy of our present analytical methods for studying these reactions.

It is the purpose of this paper to call attention to some discrepancies between the proteinase theory as proposed by Jørgensen and by Balls and Hale and the observed action of reducing and oxidizing agents in doughs. The characteristic course of the action of oxidation and reduction in doughs seems to us to furnish convincing evidence that the manifestations of reduction and of oxidation in doughs are not due to the activation and inactivation of proteolytic enzymes. It is well known that the oxidation of a dough by bromate is slow and is progressive over a considerable period of time. If doughs made from unoxidized flour are treated with normal quantities of $KBrO_3$ (1 to 2 mg %) the oxidizing action is not noticeable in the dough until after a considerable period of fermentation; there is a gradual development

of oxidation or gradual disappearance of reduced character. If the reduced character is the result of proteolytic disintegration of the protein, its disappearance should mean a resynthesis of the gluten protein. As compared with the action of $KBrO_3$, the action of KIO_3 or $NaClO_2$ is exceedingly rapid and is noticeable in the dough as it comes from the mixer (Sullivan, Howe, Schmalz, and Astleford, 1940; Baker and Mize, 1941), although even with these agents there is a pronounced increase in oxidation with time.

Though these points are readily observed in the fermentation and baking of unbleached flours and are quite generally known, apparently their significance has been overlooked in the proteolytic-inhibition theory; accordingly they are included in the experimental work reported in this paper.

Experimental

Unbleached second clear flour may be effectively used to illustrate the action of naturally occurring proteolytic enzymes and of added oxidizing agents, since the reduced character of the untreated dough and its response to oxidation are obvious and proteolytic enzymes are present in much higher concentration than in the higher-grade flours (Cairns and Bailey, 1928). Also the response to oxidation in clear flours is not complicated by the gelation of pentosans (Baker, Parker, and Mize, 1942). Accordingly, a commercial 15.8% protein second clear flour was used for the following series of demonstrations.

Baking Method

The micro baking technique used in this study is founded on that described by Van Scoyk (1939) as modified in this laboratory by Sandstedt and Ofelt (1940). The following formula was used for this particular study:

Flour 25 g (15% moisture basis)
Salt 1%
Yeast 3% (Fleischmann)
Shortening 3% (Crisco)
Water 60%

Sugar was adjusted to fermentation time so that the baked loaves had approximately the same residual sugar content. The sugar added to the second clear doughs varied from 1.5% for a 1-hour fermentation to 7.2% for a 5-hour fermentation; 8% sugar was added to the experimentally milled Turkey flour for the 4-hour fermentations (3-hour sponge and 1-hour dough).

The doughs were given an "optimum" first mix in the National micromixer. The sponge doughs were given a one-minute second mix which according to dough appearance and handling properties seemed

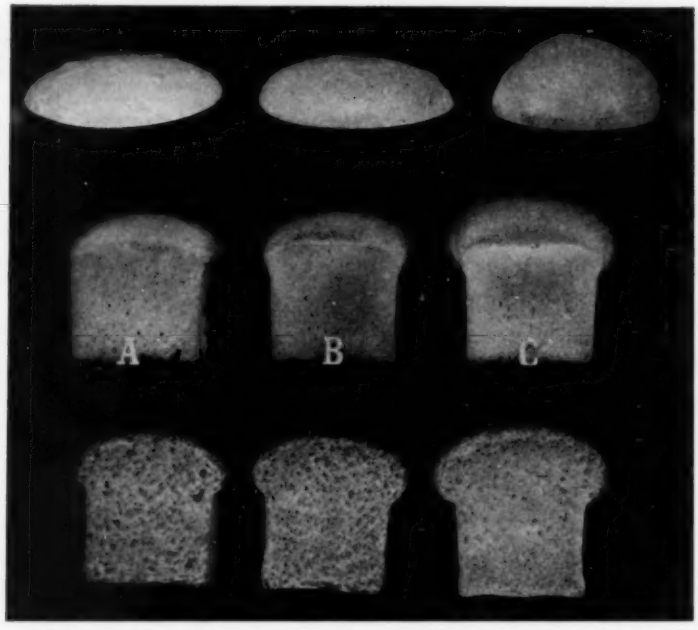

Fig. 1. Development of characteristics of oxidation during a short fermentation with unbleached second clear flour: *A* with no oxidizing agent, *B* with 2 mg % bromate, *C* with 1½ mg % chlorite.

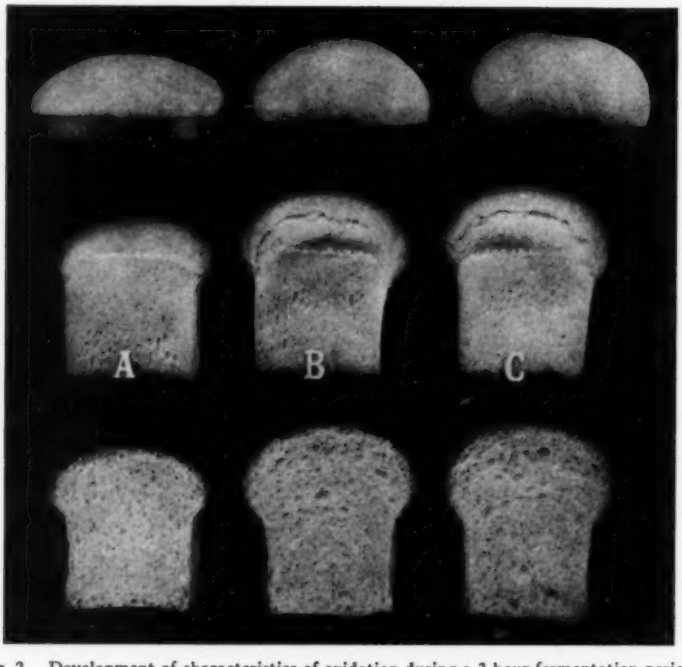

Fig. 2. Development of characteristics of oxidation during a 3-hour fermentation period. Flour and oxidation as in Figure 1.

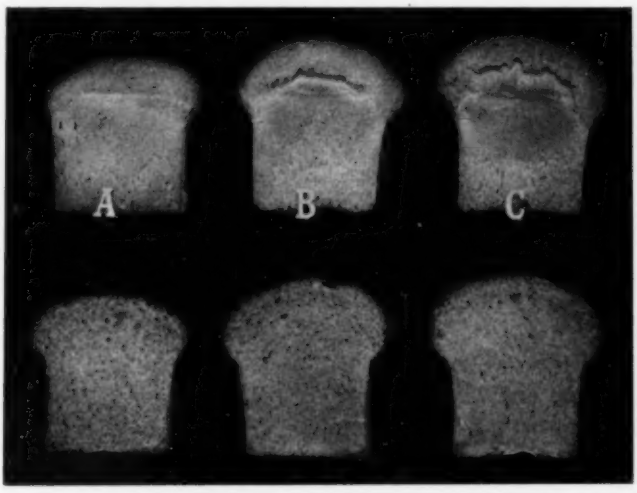

Fig. 3. Development of the characteristics of oxidation in 100% sponge doughs. Flour and oxidation as in Figure 1. Oxidizing agents added at first mix.

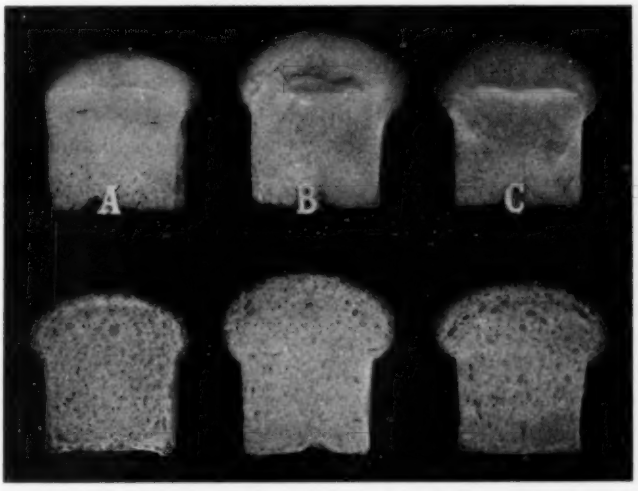

Fig. 4. Reversal of characteristics of natural reduction by oxidation. 100% sponge doughs with oxidizing agent added at the second mix. Flour and oxidation as in Figure 1.

to be "optimum" and was sufficient to give a uniform distribution of the oxidizing agent through the dough.

The doughs were punched and molded by sheeting through the National sheeter and rolling into a loose roll. Proofing was to 6.5 cm height in the tall-form pans.

The doughs and loaves shown in Figures 1 to 4 illustrate the characteristic progress of oxidation—the slow action of $KBrO_3$ and the rapid action of $NaClO_2$. The doughs of Figure 1 were photographed

after a fermentation of 1¾ hours at 30°C, and the loaves were baked from doughs having a corresponding total fermentation: 1 hour fermentation plus proof (approximately 45 minutes). Loaf *A* had no oxidizing agent added, *B* 2 mg % $KBrO_3$, and *C* 1½ mg % $NaClO_2$. It is seen that the unoxidized dough and bread had pronounced characteristics of reduction; according to the Jørgensen-Balls and Hale theory these are the results of proteinase action. However, the dough and corresponding bread oxidized by $KBrO_3$ also showed these same effects so, according to theory, the $KBrO_3$ had not appreciably inhibited proteolysis. On the other hand, the dough and bread containing $NaClO_2$ showed very marked effects of oxidization, or according to theory, lack of proteolysis.

The doughs and loaves shown in Figure 2 show the effects of fermentation for an additional 2 hours, corresponding to the usual 3-hour fermentation and proof. The doughs shown were punched at the end of the first 2 hours and then allowed to rise 1¾ hours for the dough pictures; the loaves were baked from doughs having the usual A.A.C.C. punching treatment. It is seen that the unoxidized dough and corresponding loaf still showed reduced character—though not as strongly as at 1¾ hours. The $KBrO_3$ oxidized dough and loaf now showed considerable oxidation. If these oxidized characteristics were brought about by inhibition of proteolysis, the proteinase which produced the reduced characteristics of the corresponding dough and loaf in Figure 1 (*B*) must not only have been inhibited but its action must have been reversed by the extension of fermentation. It seems highly improbable that proteolytic decomposition could be so readily reversed by oxidation. The $NaClO_2$ loaf at this stage showed evidence of overoxidation.

Since the reduced characteristics developed in the unbleached clear flour doughs were reversed by the action of bromate, it might be informative to extend the time of action of the naturally occurring proteinases and reducing agents beyond the period allowed by the slow action of the bromate. This may be conveniently accomplished by the use of 100% sponge doughs (all of the flour in the sponge), adding the oxidizing agent at the second mix.

Figure 3 shows the effect of remixing the doughs at the end of the 3-hour fermentation and giving an additional 25 minutes' recovery before molding; *i.e.*, the loaves were baked from 100% sponge doughs with a 25-minute dough time. In this case, all ingredients, including the oxidizing agents, were added at the first mix; accordingly these loaves were comparable to those shown in Figure 2. The characteristics of the loaves were not materially changed except for a finer grain in the oxidized loaves.

The loaves shown in Figure 4 were produced from 3-hour 100% sponge doughs with a 2-hour dough time, the oxidizing agent being introduced at the second mix. Loaf *A* received no oxidizing agent, *B* was given 2 mg % of bromate, and *C* 1½ mg % of chlorite. It is obvious that the 3-hour action of natural proteinases and reducing agents was completely reversed by the subsequent action of the oxidizing agents. This is a strong indication that even the relatively large quantity of proteolytic enzymes reported to be present in clear flours is of little consequence in baking.

If the reduced character of the unoxidized loaves in this series of bakes was due to proteolysis, it seems that this character should have

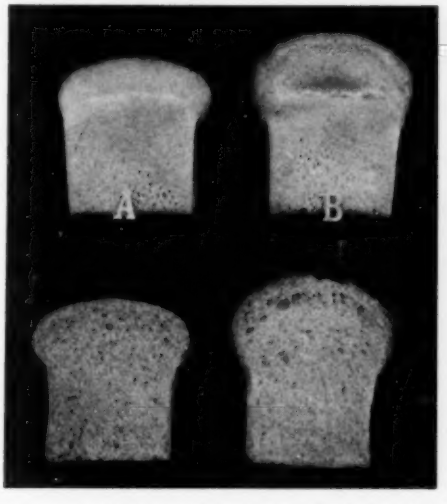

Fig. 5. Effect of subsequent oxidation on natural reduction of an unbleached high protein patent flour 100% sponge dough. *A* with no oxidizing agent, *B* with 1½ mg % $NaClO_2$ added at the second mix.

become progressively more pronounced with increased fermentation (Shen and Geddes, 1942). It is seen that instead of the reduced character becoming intensified with time of fermentation, it became less pronounced (compare loaves in Figs. 1, 2, 3, and 4) and, if the proteolysis theory were accepted, the surprising conclusion would be that there was less proteolysis during the long fermentation than during the short fermentation.

Loaf *C* (Fig. 4) showed definite indications of overoxidation. Freilich and Frey (1939) point out that the action of bromate "goes beyond any point at which it might be explained as due to retardation of proteolytic activity." Certainly it seems strange that a deficiency of proteolysis may be created in a dough which had undergone too much proteolysis before the inhibiting agent was introduced.

These studies involved only the naturally occurring proteolytic enzymes and naturally occurring reducing agents of flour. To throw more light on the action of reducing agents and proteolytic enzymes in doughs, further studies were made to compare the action of added papain and reducing agents. A composite of a number of high-protein, experimentally milled, unbleached patent Turkey wheat flours was used for this study. A high protein (14%) was chosen in order to obtain unmistakable differences in the character of loaves produced by the various treatments, and 100% 3-hour sponge doughs were used so that reducing agents or papain could be added at the

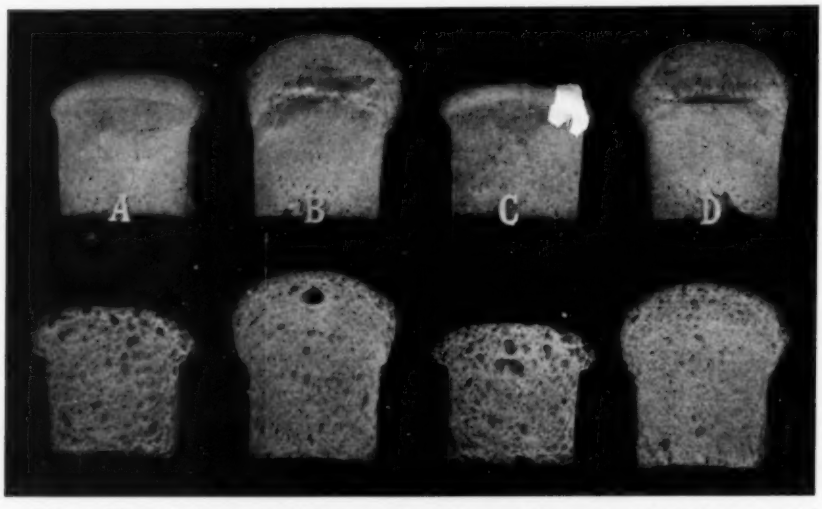

Fig. 6. Effect of subsequent oxidation on reduced characteristics produced by Na_2SO_3. *A* and *B* given 12 mg % Na_2SO_3, and *C* and *D* given 16 mg % Na_2SO_3 at the first mix; *B* and *D* given optimum oxidation at the second mix. Compare with Figure 5.

first mix and allowed to act on the dough for a period of time before the oxidizing agent was introduced. A 1-hour dough time was allowed after the second mix, giving the oxidizing agent (added at the second mix) one hour plus the proof time in which to act.

The loaves shown in Figure 5 were the check loaves for this series of bakes. Loaf *A*, being the negative check, had no reducing or oxidizing agents added. Loaf *B* was given optimum oxidation at the second mix. This is the standard for comparison of all optimally oxidized loaves produced by various treatments. The optimum oxidation was determined by a series of bakes, varying the $NaClO_2$ added by 0.5 mg % steps; the loaf showing the greatest volume combined with good characteristics was taken as the optimum.

Figure 6 shows the effect of allowing a reducing agent to act on the doughs for a 3-hour period. Twelve mg % Na_2SO_3 was added at

the first mix to the doughs from which loaves *A* and *B* were baked. *A* received no oxidizing agent at the remix while *B* received 4 mg % $NaClO_2$ (optimum). Loaves *C* and *D* were given 16 mg % Na_2SO_3 at the first mix; *C* no oxidation; *D* 5 mg % $NaClO_2$ at the remix. The increase in reduced character produced by the Na_2SO_3 may be seen by comparing loaves *A* and *C* with loaf *A* of Figure 5. That the effects of the sulfite addition were reversed by the subsequent oxidation may be seen by comparing loaves *B* and *D* with loaf *B* of Figure 5; seemingly the action of the added reducing agent had produced no irreversible detrimental effects.

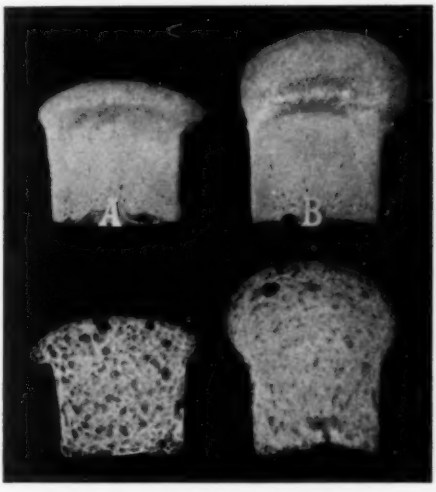

Fig. 7. Reduction with 16 mg % glutathione, with subsequent oxidation. *A* with no oxidation *B* with 5 mg % $NaClO_2$ at second mix. Compare with Figure 6.

The characteristics produced by glutathione are similar to those produced by reduction with Na_2SO_3 and are also reversible by oxidation. This similarity is illustrated by the loaves shown in Figure 7. These were baked from dough which had been treated with 16 mg % glutathione at the first mix. Loaf *A* was the unoxidized check while loaf *B* was oxidized with 5 mg % $NaClO_2$ at the second mix. Under these baking conditions the 16 mg % of glutathione had a reducing action comparable to 16 mg % Na_2SO_3 (loaf *C*, Fig. 6). This approximately equal quantitative relationship would not be expected to hold for other samples of glutathione with more reduced or less reduced character. However, on oxidation the glutathione-treated dough (loaf *C*, Fig. 7) produced a larger loaf than could be produced by oxidation of sulfite-treated doughs (loaf *C*, Fig. 6) or by oxidation of the untreated dough (loaf *B*, Fig. 5), indicating that oxidized gluta-

thione might be a better dough improver than some other oxidizing agents (Ziegler 1940).

The above experiments have shown that reduction in doughs may be reversed by oxidation and that the naturally occurring proteolytic enzymes of a second clear flour produced no perceptible irreversible degradation. How does the action of added papain (added in quantity to produce the same reduced appearance in the bread as was produced by the reducing agents used in the above experiments) compare with these observations?

The loaves shown in Figure 8 were all produced from doughs in which papain was added at the first mix. In preliminary experiments it was found that the addition of 2.0 mg % of the particular sample

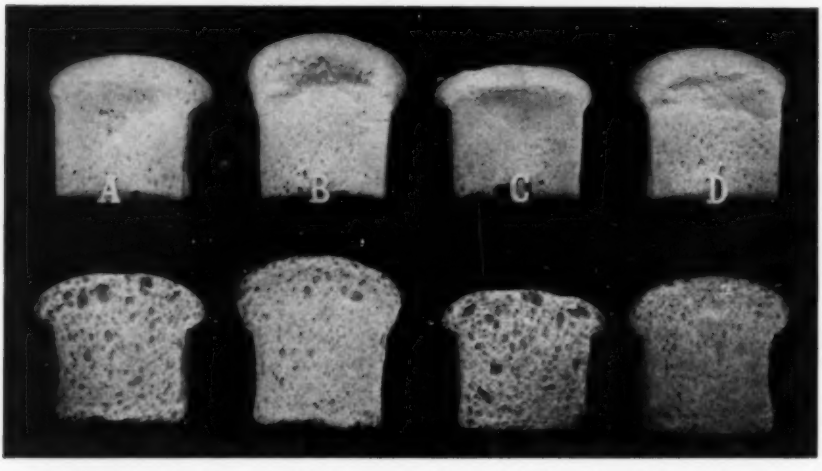

Fig. 8. Effect of subsequent oxidation on papain treated doughs. *A* and *B* were given 2 mg % and *C* and *D* 3 mg % papain at the first mix. *B* and *D* were given optimum oxidation at the second mix. Compare with Figure 6.

of papain which we were using produced loaf characteristics similar to those obtained from the use of 12 mg % of Na_2SO_3 and that 3.0 mg % produced characteristics similar to those produced by 16 mg % of Na_2SO_3. Loaves *A* and *B* of Figure 8 were produced from doughs which had been treated with 2 mg % of papain at the first mix, loaf *A* being the check which was not oxidized while loaf *B* was given 4 mg % $NaClO_2$ at the remix. Loaves *C* and *D* were from doughs treated with 3 mg % papain at the first mix, loaf *C* being the check with no oxidation while *D* was given 5 mg % $NaClO_2$. Loaves *B* and *D* were the largest loaves that we were able to produce by subsequent oxidation from these papain-treated doughs. By comparing loaf *A* of Figure 6 with loaf *A* of Figure 8, and loaf *C* of Figure 6 with loaf *C* of Figure 8, it may be noted that the papain-treated doughs gave characteristics that

could easily lead one to conclude that proteolysis and reduction were identical in effect. However, it is seen that the proteolysis which occurred in the papain-treated loaves could not be reversed by oxidation; *i.e.*, though the characteristics due to reduction and proteolysis appeared to be quite similar, the reduction could be reversed while the proteolysis could not. Comparing loaves *B* and *D* of Figure 8 to loaf *B* of Figure 5, it is seen that papain caused a loss in the maximum volume obtainable from this flour by oxidation.

While proteolysis and reduction produce characteristics in doughs and bread that are visually indistinguishable, they have a decidedly different action on the gluten proteins. Since no perceptible irreversible effect of proteolysis was found in the second clear doughs, we conclude that even the relatively large quantity of naturally occurring proteolytic enzyme present in the second clear flour (as compared to that present in normal patent or straight flours) was present in such small amounts as to be of no significance in baking.

Summary

The action of the reducing agents naturally occurring in flour may be reversed by subsequent oxidation. This is considered as evidence that the reduced character of doughs and bread made from unoxidized flour is not due to proteolysis. Moreover it indicates that even the relatively large quantity of proteinase reported to be present in clear flours (as compared to normal patents and straights) produces no perceptible irreversible degradation.

The action of reasonable quantities of added reducing agents is also shown to be reversible by subsequent oxidation. Degradation of the gluten proteins by papain (added in quantity to produce similar reduced appearance in the bread as was produced by the added reducing agents) was not reversible by subsequent oxidation. Accordingly, though proteolysis and reduction produce characteristics in doughs and bread that are visually indistinguishable, they do not have identical modes of action on the gluten.

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ANGEL FOOD CAKES MADE FROM FRESH AND FROZEN EGG WHITES[1]

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(Received for publication January 15, 1943)

Increased production of frozen eggs for human consumption during the last few years indicates a growing interest in their use. Prescott and Proctor (1937) have predicted that people now living will see the day when the housewife will buy frozen eggs for most culinary uses. Improved methods of freezing and storing these eggs have aided greatly in making them available for general use.

Sweetman (1937) states that the nutritive value of eggs does not change with freezing, and experiments show frozen eggs to be as satisfactory as fresh eggs in most food mixtures. Bakers, food manufacturers, and others who use large quantities of eggs have found the frozen product more convenient to use, more uniform in quality, better adapted to large-scale usage, and more economical, on the whole, than fresh eggs.

The quality of frozen eggs depends upon the fresh eggs from which they are made, upon the method of freezing, and the care taken in the freezing and storing of the finished product. Frozen eggs retain their

[1] Contribution No. 113 N. S., School of Home Economics, Kansas State College of Agriculture and Applied Science. Condensed from the thesis presented by Elsie Lee Miller in partial fulfillment of the requirements for the Degree of Master of Science, May, 1942.

original quality for long periods of time if held at sufficiently low temperatures, but special care must be exercised during and after thawing. LeClerc and Bailey (1940) recommended that frozen whites be held at refrigerator temperature until entirely thawed, then thoroughly mixed to combine the solids and the fluids.

Lowe (1940) found that angel food cakes made from frozen whites were as good in every respect as those made from fresh whites. Although considerable work has been done on the whipping of fresh egg whites and their use in angel food cakes and some work has been done with frozen whites, there are still numerous unanswered questions. Commercial producers of frozen eggs may or may not treat the egg whites prior to freezing, to alter their viscosity. Some companies are marketing both thick and thin frozen whites. In recent years considerable interest has been shown in the relative whipping quality of thick and thin egg whites and studies have been made of these two types, both as obtained by separating the individual whites into the two portions and by using eggs of various ages.

Egg whites, when beaten, form a foam which consists of many small air bubbles surrounded by protein film. The ability of egg whites to form a foam is due to the low surface tension of the whites and to the stability of the surface films. When egg whites are beaten the coagulation of the protein is one of the results of the mechanical agitation and it is this which gives rigidity to the foam structure.

Rahn (1932) stated that in order to have a stable foam it is necessary to have in the solution colloids which concentrate in the surface as a result of the lowering of surface tension and then solidify readily. He believed that egg albumin behaves like other typical foam substances and conforms to the accepted theories of foam stability.

The authors of *Eggs and Egg Products* (U. S. Dept. Agr., 1941) observed that the whipping quality of egg whites seemed to vary with the viscosity or with the quality of fluid white that they contain. The more viscous the white, the longer it took to form a foam. The resulting foam had less volume but greater stability than that formed from thinner whites.

St. John and Flor (1931) stated that the very firm white took longer to beat because it was apparently necessary to break up the gross colloidal structure before air could be incorporated. The resulting foam not only had less volume but was also more pasty and less fluffy than foams obtained with thinner whites.

Almquist and Lorenz (1932) suggested that the firm white is composed of a fine, fiber network of pure ovomucin in which is entrapped the ordinary liquid white. As the carbon dioxide escapes, the fibers break up and can no longer entrap the liquid white. These workers

(1933) considered the difference between firm and liquid whites to be one of structure and not of water content, and Holtz and Almquist (1931) showed that the total solids content of the thin egg white is equal to that of the firm white.

St. John and Flor (1931) and others have stated that a low viscosity increases the ease with which a foam may be formed but Barmore (1934) demonstrated that "the specific gravity of the foam was directly proportional to the viscosity of the egg white draining from that foam." Barmore believed that "as the viscosity is reduced the beater is able to tear the liquid into thinner and thinner liquid layers that then entrap the air." This worker also observed that the decrease in viscosity resulting as the period of beating increased was further evidence that the thick egg white is composed of a fine fiber network of protein, entrapping the ordinary liquid and that beating probably tears up the fibers and destroys the structure.

St. John and Flor (1931) recommended that egg whites be at a temperature of 60° to 70°F before beaten and workers in the Wilson and Company laboratories (1939) found that the best foams were produced at whipping temperatures of 70° to 80°F. St. John and Flor (1931) believed their results with different beating temperatures were due, at least in part, to the effect of temperature upon the consistency of the whites. They found that both the thick and thin portions of the white were relatively thicker at lower temperatures and that air was incorporated with greater difficulty. The statement is made in *Eggs and Egg Products* (U. S. Dept. Agr., 1941) that "the temperature of the egg white affects the speed with which the foam is formed and the volume obtained." The reason given is that with an increase in temperature there is a decrease in the surface tension of the egg whites; the result is that eggs at room temperature beat more readily than those taken directly from the refrigerator.

The purpose of this study was to determine the best temperatures for whipping fresh and frozen whites for angel food cakes, to determine the most satisfactory baking temperature for cakes made from these different whites, and to compare the quality of angel food cakes made from fresh and frozen eggs.

Method

The work was divided into two series of experiments. In Series I, a study was made of the effect of different beginning beating temperatures of the egg whites on the quality of angel food cakes. In Series II, the effect of different baking temperatures was determined.

Series I: Sixty angel food cakes were made with 4 beginning beating temperatures: 40°, 50°, 60°, and 70°F. Five cakes were made with

each of the beginning beating temperatures from each of three kinds of egg whites. In order that the results might be carefully checked, six cakes were baked each day, two from each of the three types of egg whites. All cakes of the first series were baked at 350°F for 40 minutes.

Series II: Seventy-five cakes were baked at 5 different baking temperatures and times: 350°F for 40 minutes, 375°F for 35 minutes, 400°F for 30 minutes, 425°F for 25 minutes, and 450°F for 21 minutes. The same three types of egg whites were used as in Series I. Five cakes made from each of the three types of egg whites were baked at each of the five baking temperatures.

The ingredients for each series were as nearly identical as possible. The only ingredient varied throughout the study was the egg whites. The three types of egg whites used were: No. 1 fresh, thick frozen, and thin frozen.

A recipe and a method of mixing angel food cake on an electric mixer, developed at Kansas State College, were used. The cakes were baked in ovens equipped with thermostatic heat controls.

The following determinations were made: temperature of the foam and batter; specific gravity of the foam and batter (Platt and Kratz, 1933); volume of batter; area of a slice of cake as an index of volume (Platt and Kratz, 1933); percentage loss of weight during baking; compressibility, elasticity, and tenderness of the cake (Platt and Kratz, 1933); and the quality as judged by a palatability committee.

Results and Discussion

A summary of the results using different beginning beating temperatures is given in Table I. All three types of egg whites were found

TABLE I

MEAN RESULTS FOR CAKES MADE FROM EGG WHITES OF DIFFERENT TEMPERATURES (SERIES I)

Temperature of egg white	Volume of batter	Area of slice of cake	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
°F	*cc*	*sq cm*	*mm*	*mm*	*g*	*cm*	
40	2506	73.9	9.3	2.5	67.2	4.4	49
50	2555	77.1	9.5	2.6	65.1	4.2	48
60	2651	80.2	9.3	2.8	67.5	4.3	49
70	2788	80.7	8.6	2.9	62.4	4.4	51

[1] Possible score, 70 points.

to whip better at 70°F than at other temperatures tried. Whites whipped at this temperature produced good foams which remained quite stable. All of the cakes were more tender and of better texture when they were made from whites whipped at 70°F. Cakes made from

whites whipped at 40°F were more compact and much smaller in volume than those from whites whipped at the higher temperatures. Some difference in color was noted in the cakes made from whites of different beginning beating temperatures. When the whites were at 70°F before beating, the resulting cakes were more nearly white than those made from the same kinds of whites whipped at lower temperatures. The results obtained agree with those of other workers who, as has already been discussed, have shown that up to a certain point an increase in temperature resulted in improved whipping quality of both thick and thin egg whites. This may be due to the fact that as the temperature increases up to 70° or 80°F, the whites become thinner and the surface tension decreases.

The thin frozen whites whipped more quickly than the thick frozen ones, but both types whipped more quickly than the fresh whites. There appeared to be a greater amount of leakage from the foams of the fresh than from either type of frozen whites. There was little leakage from the foams of the thin frozen whites when whipped at 70°F. The average results for the cakes made from the three types of egg whites in Series I are given in Table II. At all four beginning

TABLE II

MEAN RESULTS FOR CAKES MADE FROM THREE TYPES OF EGG WHITES (SERIES I)

Type of egg whites	Area of slice of cake	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
	sq cm	*mm*	*mm*	*g*	*cm*	
Fresh	75.4	9.3	2.7	63.9	4.3	49
Thick frozen	75.8	8.4	2.8	76.1	4.0	49
Thin frozen	82.7	10.0	2.6	56.8	4.9	49

[1] Possible score, 70 points.

beating temperatures the thin frozen whites produced the greatest volume of finished batter and the largest baked cake, but the difference was greatest when the whites were beaten at 70°F. Cakes made from the thick frozen whites had the second greatest volume and those from the fresh whites slightly less.

A possible explanation for the results with thin frozen whites may be found in Barmore's work (1934), which showed that beating tears up the fiber structure of the egg white, thus reducing the viscosity at the same time that the foam is being formed. And Almquist and Lorenz (1932) have shown that in the absence of sufficient CO_2 the fibers of the firm white break up, thus giving a thinner white. It seems probable then that thin frozen whites resulting from mechanical treatment or from changes brought about by allowing the egg whites to

"age" either by natural or artificial methods would beat to a greater volume and in less time than would the more viscous whites.

The results, obtained with various baking temperatures, are summarized in Table III. Volume, as indicated by the area of a slice of cake, increased with increase of baking temperature up to 425°F. At 450°F there was a definite loss of volume. As is shown in Table III, the cakes baked at 400°F had the greatest compressibility and were given the highest score by the palatability committee but were definitely less tender than some of the other cakes. Cakes baked at 425°F were most tender and rated high both in compressibility and in palatability score. There was a decrease in the percentage loss in weight in baking with the increase in baking temperature. Cakes baked at 350°F lost 43.1% in weight, whereas those baked at 450°F lost only 30.6%. This was evident in judging the cakes, for those baked at the higher temperatures were more moist; in fact, many of the cakes baked at 450°F were so moist as to be almost soggy. There was no great variation in the color and flavor of the cakes baked at the different temperatures.

TABLE III

MEAN RESULTS FOR CAKES BAKED AT DIFFERENT TEMPERATURES (SERIES II)

Baking temperature	Area of slice of cake	Percentage loss during baking	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
°F	*sq cm*	%	*mm*	*mm*	*g*	*cm*	
350	82.5	43.1	9.0	4.0	68.1	4.3	49
375	84.5	36.7	9.0	3.8	72.6	4.2	48
400	87.7	33.3	9.3	3.9	71.7	4.5	51
425	90.9	32.9	9.1	3.6	63.4	4.6	50
450	83.1	30.6	8.6	3.2	66.7	4.5	44

[1] Possible score, 70 points.

The results obtained with different baking temperatures would appear to substantiate the statement by Barmore (1935) that higher baking temperatures for angel food cakes do not decrease the tenderness because the internal temperatures of the cakes change very little with changes in baking temperatures. Lowe (1937) and Burke and Niles (1936) have reported that better cakes were produced when baked at 350°F as compared with 325°F, a temperature often recommended but lower than any used in this study. At the higher temperature these workers obtained larger cakes, which were more moist and tender. Work in the laboratories of Wilson and Company (1939) indicated that temperatures of 425°F to 450°F produced angel food cakes of better quality and greater volume than cakes baked at lower temperatures.

Table IV shows that in Series II, as in Series I, the thin frozen whites produced cakes of greatest volume, compressibility, and tenderness. In Series II the score of the palatability committee indicates a preference for the cakes made from the fresh egg white. This was in

TABLE IV

MEAN RESULTS FOR CAKES MADE FROM THREE TYPES OF EGG WHITES (SERIES II)

Type of egg whites	Area of slice of cake	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
	sq cm	*mm*	*mm*	*g*	*cm*	
Fresh	85.5	8.9	3.6	70.2	4.4	50
Thick frozen	84.0	8.8	3.8	73.9	4.3	48
Thin frozen	88.0	9.4	3.8	61.8	4.5	48

[1] Possible score, 70 points.

part due to the more acceptable flavor of these cakes. This difference was not noted in the cakes of the first series, which were made a year before but which used the same brand and grade of whites.

Summary

A beginning beating temperature of 70°F and a baking temperature of 400°F for 35 minutes or of 425°F for 30 minutes produced the most desirable angel food cakes with fresh, thin frozen, and thick frozen egg whites. Both the thin frozen and the fresh whites produced cakes of high quality with similar characteristics but those made from the thick frozen whites were less desirable.

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CHEMICAL FACTORS AFFECTING THE BAKING QUALITY OF DRY MILK SOLIDS. III. THE EFFECT OF SEVERAL MILK FRACTIONS ON LOAF VOLUME[1]

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(Received for publication April 21, 1943)

The baking quality of dry milk solids has been greatly improved as the result of the investigations of Greenbank *et al* (1927), Grewe and Holm (1928), and Skovholt and Bailey (1931). These workers found that the baking quality of the dried product was enhanced by heat treatment of the fresh skim milk. Heat treatment is now a general practice when the dry milk solids are to be used in bread making.

Many studies have been made on the effect of heat treatment on the baking quality of dry milk solids. If the reason for the improvement in baking quality, following heat treatment, could be established and associated with a definite fraction of the skim milk, it might aid in determining what factors are responsible for the variability in the baking quality of heat-treated dry milk solids.

Stamberg and Bailey (1942) used the Heyrovsky micropolarograph to show that unheated fresh skim milk contains sufficient sulfhydryl groups to cause definite softening of dough. They found that boiled milk had no measurable amount of sulfhydryl groups. The dough-softening factors in the unheated fresh milk were in the whey portion, as shown by means of the farinograph. The tests were made on wheys prepared from fresh skim milk by both the acid and rennet

[1] Scientific Paper No. 554, College of Agriculture and Agricultural Experiment Station, State College of Washington.
[2] American Dry Milk Institute Research Grant and in cooperation with the Washington State Dairy Products Commission.

precipitation methods and the same results were obtained in each case. However, no baking tests were reported on the various fractions of fresh skim milk or dry milk solids.

The goal of this problem is to find the fraction or fractions of the skim milk which are responsible for variations in baking quality. Were such found, it appears logical to expect that a test could be devised that would replace the present slow and relatively uncertain baking test for the elimination of the few lots of poor-baking dry milk solids now being made.

Materials and Methods

The dry milk solids were obtained from certain Northwest Washington plants that use the spray process. The baking quality of these samples was determined for commercial grading at the baking laboratory of the Consolidated Dairy Products Co., Seattle, Washington. The samples were selected to represent good and poor baking. Fresh skim milk was obtained from a Grade A supply of pasteurized milk. Both unheated and heat-treated skim milk were used. In the early work (Table IV), a heat treatment of 82°C for 45 minutes was used, and in the remainder of the work the heat treatment was 90–95°C for 5 minutes. The milk fractions were all prepared from the above-mentioned source of milk.

Acid whey was prepared by the slow addition of dilute hydrochloric acid (1 volume of concentrated cp HCl in 9 volumes of water) to rapidly stirred skim milk at 35°C until a pH of 4.65 was recorded by a meter equipped with a glass electrode. The mixture was left for 30 minutes to precipitate and the whey decanted through a filter paper. The filtrate was then adjusted to pH 6.5 with 4*N* sodium hydroxide. When concentrated whey was used, the technique of Palmer (1934) was followed. Rennet whey was prepared by the addition of 0.2 ml of Hansen's cheese rennet to 750 ml of the skim milk at 35°C. The milk was left for 30 minutes to clot and the curd cut to facilitate the expulsion of the whey. The curd was then strained off and the whey filtered to remove fine particles.

Casein was prepared for the baking tests by acid precipitation from fresh skim milk, also at pH 4.65. The separated curd was washed twice by decantation with an excess of cold water and redispersed in water by the addition of a minimum amount of 4*N* sodium hydroxide. The casein was immediately reprecipitated, washed as before, and again dispersed with the sodium hydroxide in about half the amount of water. The alkaline casein solution was immediately cooled to about 0°C and the pH adjusted to 6.5 by the slow addition of the previously mentioned dilute hydrochloric acid while the solution was

rapidly agitated with an electric stirrer. While the casein solution was held at about 0°C, the nitrogen concentration was determined and the amount of casein in the solution calculated for the baking test. The sodium chloride concentration of this solution was determined in order that the necessary corrections could be made on the salt used in the baking formula.

For dialysis of the milk and milk fractions, a cellophane tubing $1\frac{1}{8}$-inches in diameter, obtained from the Central Scientific Company, was found to be satisfactory. The dialysis apparatus was similar to the equipment described by Lampitt and Bushill (1933). The cellophane tubing, tied at one end and mounted in rubber at the other, was suspended in a specially designed glass cylinder so that distilled water could be rapidly circulated around the dialysis sac. The dialysis was complete in 48 hours as shown by the absence of reducing sugars.

TABLE I

FORMULAS AND BAKING METHODS

	Method 1: Hard-wheat, highest patent, high-baking-strength flour	Method 2: Hard-wheat, highest patent, medium-baking-strength flour
Formula		
Flour	100 g	100 g
Salt (NaCl)	1.5	1.5
Sucrose	3.5	4.0
Yeast	3.0	3.0
Shortening	0.0	2.0
Water and milk fractions	Variable	Variable
Mixing time (Swanson mixer)	2 min	2 min
Fermentation time	180 min (30°C)	125 min (30°C)
Proofing time	55 min (30°C)	55 min (30°C)
Molding methods[1]	Hand (3rd ed. CLM)	Hand (4th ed. CLM)
Type of pans	Low form	Low form
Baking time	25 min (232°C)	25 min (232°C)
Weight per loaf	150 g dough	90 g flour

[1] CLM refers to *Cereal Laboratory Methods*.

Since a concentrated dialysate of the skim milk could not be recovered, an ultra-filtration method was used to obtain the dialysate material. For this work a suitable length (approximately 21 cm) of the cellophane tubing was mounted in rubber and placed in a liter suction flask. The air pressure in the flask was reduced by approximately 100 mm of mercury and the distended sac filled with the milk to be filtered. The ultra-filtrate was obtained during 48 hours. The ultra-filtration and dialysis, with the exception of the work shown in Table VI, was done in a cold room at 4°C to prevent fermentation. The high lactose content of the concentrated whey made it desirable to dialyze it against tap water at 15°C for 24 hours before completing the dialysis another 24 hours at 4°C.

The baking methods followed were, in the main, those outlined in *Cereal Laboratory Methods* (1935, 1941), but certain modifications were necessary. The details of the methods employed are given in Table I and the method used is stated in each table concerned.

Experimental

The data in Table II show the amount of nitrogenous substances found in several of the fractions used for baking. The results show that the ultra-filtrates from fresh skim milk and dry skim milk each have a nitrogen value similar to that of the corresponding dialysate. Because of the removal of approximately 78% of the nitrogen by the

TABLE II

NITROGEN DISTRIBUTION IN MILK FRACTIONS

Milk fraction	Percent of total nitrogen			
	Non-dialyzable	Dialyzable (by difference)	Ultra-filtrate	Rowland's (1938) for nonprotein
Dry milk solids	94.0	6.0	5.5	—
Fresh skim milk	95.2	4.8	5.1	5.0
Fresh skim milk whey (acid method)	77.5	22.5	—	23.0

acid precipitation of the casein from the whey, the dialyzable fraction of the whey would be expected to be about five times as great as that from the fresh skim milk. The data show that the distribution of nitrogenous substances in the whey is approximately in this proportion. The values in Table II were not determined from a sufficient number of samples to assure representation of all milks, but they are representative of this work. It may be noted that Rowland's (1938) values for nonprotein nitrogen are approximately the same as those from the dialyzable fraction. The ultra-filtrate from both the dry milk solids and the fresh skim milk failed to give a positive Biuret test for proteins. Thus it is evident that little if any proteose or peptone material passed through the cellophane membrane.

The average volumes of the loaves made from samples of good and poor dry milk solids are shown in Table III. The results show a highly significant difference in loaf volume between those that baked well and those that baked poorly. The dialyzable fractions from good and poor dry milk solids, when used in the bread formula, produce loaves of practically the same volume. The poor-baking factor, which accounts for the low loaf volumes, is thus shown to be in the non-dialyzable fraction. The extent of the reduction in volume of the loaves by the nondialyzable fraction over that of the dialyzable fraction

for good and poor milks is 14 and 52 cc, respectively. This is further evidence that the poor-baking factor is definitely associated with the nondialyzable fraction.

TABLE III

A Comparison of the Average Volumes of the Loaves Baked (Method 1) from the Good and Poor Baking Dry Milk Solids (DMS)

Milk fraction	Good milks [1]	Poor milks [1]	Difference
	cc	cc	cc
6% DMS	603 ± 5.6	579 ± 5.0	24*
(a) Nondialyzable fraction of DMS	596 ± 4.1	562 ± 4.7	34*
(b) Dialyzable fraction of DMS	610 ± 6.4	614 ± 4.9	4
(b-a)	14	52*	

[1] Average of four milks, duplicate bakes, 2 loaves per bake.
* These values lie beyond the 1% level of significance.

The effects of heat treatment on the volumes of loaves baked with fresh milk fractions are shown in Table IV. Heat treatment resulted in a highly significant increase of 56 and 51 cc for the volumes of the controls and nondialyzable fraction, respectively. The heat treatment had practically no effect on the dialyzable portion of the milk. The poor-baking quality is again shown to be in the nondialyzable fraction.

TABLE IV

Effect of Heat Treatment on the Volumes of Loaves Baked (Method 1) with Fresh Milk Fractions

Milk fraction	No. of bakes	Unheated	No. of bakes	Heated	Difference
		cc		cc	cc
Skim milk (controls)	9	576 ± 2.3	4	632 ± 4.7	56*
Nondialyzable fraction	3	555 ± 5.0	4	606 ± 5.5	51*
Dialyzable fraction	4	697 ± 2.7	4	689 ± 3.6	8

* These values lie beyond the 1% level of significance.

The data thus far presented show that the poor-baking factor of the milk is associated with the nondialyzable fraction. The nondialyzable fraction contains the casein, lactalbumin and lactoglobulin, the insoluble salts, enzymes, and cellular constituents. The nondialyzable fraction of the skim milk was divided as follows:

Rennet whey—casein precipitated from the skim milk by rennet at pH 6.6.
Acid whey—casein precipitated by acid at pH 4.65.
Casein, precipitated from the skim milk by acid, which can be washed and redispersed by sodium hydroxide.

The removal of the soluble materials from the whey fractions was obtained by dialysis. The effect of including these fractions in the baking formula was studied.

The baking quality of acid and rennet wheys, both heated and unheated, is shown by the results of the baking tests in Table V. These results show a highly significant heat-labile factor in both the rennet and acid wheys, which appears slightly greater in the former.

TABLE V

EFFECT OF HEAT TREATMENT ON THE VOLUME OF LOAVES BAKED (METHOD 1) WITH ACID AND RENNET WHEYS FROM FRESH SKIM MILK

Milk fraction	No. of bakes	Unheated	No. of bakes	Heated	Difference
		cc		cc	cc
Rennet whey	3	578 ± 6.7	3	632 ± 6.8	54*
Acid whey	3	577 ± 7.5	3	619 ± 3.6	42*

* These values lie beyond the 1% level of significance.

However, these data show no significant difference between the two wheys. Concentrated whey, both dialyzed and undialyzed, was tested for its effect on baking. The results, shown in Table VI, show a highly significant heat-labile factor present, as was the case with

TABLE VI

EFFECT OF CONCENTRATION AND DIALYSIS ON THE VOLUMES OF LOAVES BAKED (METHOD 2, WITH FRESH SKIM MILK), ACID WHEY EQUIVALENT TO 14% OF THE SKIM MILK

Milk fraction whey concentrate	No. of bakes	Unheated	No. of bakes	Heated	Difference
		cc		cc	cc
Undialyzed	8	746 ± 5.4	8	818 ± 5.2	72*
Dialyzed	7	756 ± 5.7	8	802 ± 7.7	46*

* These values lie beyond the 1% level of significance.

the acid whey (Table V). It is possible that dialysis removed a part of this factor, because the increase in volume by heat treatment of the undialyzed acid whey was about one-half greater. This was not associated with pH, since the acid in the undialyzed whey was neutralized by sodium hydroxide. Since the dialyzed concentrate required

TABLE VII

EFFECT OF HEAT ON THE VOLUME OF LOAVES BAKED WITH ACID CASEIN FROM FRESH SKIM MILK

Casein equivalent to using skim milk	Baking method	No. of bakes	Unheated	Heated	Difference
%			cc	cc	cc
6	1	6	768 ± 5.4	784 ± 7.5	16
8	2	7	760 ± 3.1	773 ± 3.6	13

longer to prepare, this difference may have been associated with some other factor.

Casein prepared by the previously stated method was added to the baking formula in two quantities. The results in Table VII show that the heat-labile factor was largely absent in the casein prepared from skim milk by this method.

Discussion

This work confirms the conclusion reached by Stamberg and Bailey (1942), that the heat-labile factor, detrimental to baking, is in the whey fraction. However, the present work shows in addition that it is the nondialyzable heat-labile fractions of the acid and rennet wheys that are detrimental to baking. This is not in full agreement with Stamberg and Bailey's (1942) statement that the factor is dialyzable, but this lack of agreement could easily be due to differences in the membranes used. The detrimental heat-labile nondialyzable fraction of the whey is a small fraction of the milk and therefore might be associated with the noncasein nitrogen, the natural enzymes of the milk, or the cellular constituents.

Summary

The nondialyzable fractions of both poor-baking dry milk solids and fresh skim milk showed definite improvement in baking quality following heat treatment.

The baking qualities of both acid and rennet wheys from fresh skim milk were improved by heat treatment. The addition of concentrated whey to a dough formula further reduced the loaf volume, which was again improved by heat treatment. There was some evidence that during dialysis the detrimental factor in whey either slowly dialyzed or changed in some other manner to reduce its potency.

Casein prepared by the acid method was not changed in its influence on baking by heat treatment.

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THE THIAMIN CONTENT OF CANADIAN HARD RED SPRING WHEAT VARIETIES [1]

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(Received for publication December 28, 1942)

The thiamin content of wheat and wheat products is creating considerable interest among nutritionists, and this interest is being reflected to millers and crop men. To gain further knowledge on the thiamin content of Canadian hard red spring wheat, determinations were made on numerous samples of the principal varieties grown in Western Canada to determine the effect of variety and environment on these samples, and to supplement information published by the authors in a former paper (Jackson and Whiteside, 1942). Johannson and Rich (1942) published data on 265 commercial hard red spring wheats from most of the crop districts in Western Canada, and found a range of 2.2 to 8.0 μg of thiamin per gram (333 to 1210 International Units per pound) with an average of 3.93 μg per gram (594 IU per pound). Jackson and Whiteside found a range of 525 to 747 IU per pound for large bulk commercial lots of 1940 crop wheat. Nordgren and Andrews (1941) presented evidence that the thiamin content of wheat is influenced by wheat type, variety, and environment. For certain Canadian samples they reported a range from 1.45 to 2.81 mg per pound (483 to 937 IU per pound). Numerous authors have demonstrated that wide variations occur in different samples of wheat, but little is known about the factors that contribute to these variations.

In the studies reported here, thiamin determinations were made in the laboratory of the Paediatrics Department of the University of Toronto by the thiochrome method with the same technique referred to in the previous publication by the authors (1942).

[1] Contribution from the Cereal Division, Dominion Department of Agriculture, Ottawa, and the Department of Paediatrics, University of Toronto.
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Composited Samples of Pure Varieties

The authors (1942) published thiamin values for Canadian hard red spring wheat representing the crops of 1939 and 1940 and included data for commercial samples as well as those from plot tests of pure varieties. In Table III of that paper the thiamin content of six

TABLE I

THIAMIN CONTENT OF HARD RED SPRING WHEAT VARIETIES IN INTERNATIONAL UNITS PER POUND OF WHEAT

Variety	Year 1939	Year 1940	Mean
	IU per pound		
Apex	670	695	683
Marquis	645	660	653
Regent	820	838	829
Renown	820	792	806
Reward	806	784	795
Thatcher	700	713	707
Mean	744	747	746

varieties was given on a composite of each variety. These were made up from samples collected from plots grown at 158 places in the three Prairie Provinces in 1939. A similar set of samples was made available through the courtesy of the Crop Testing Plan for the 1940 crop. A comparison of the thiamin contents of six varieties for the year 1939

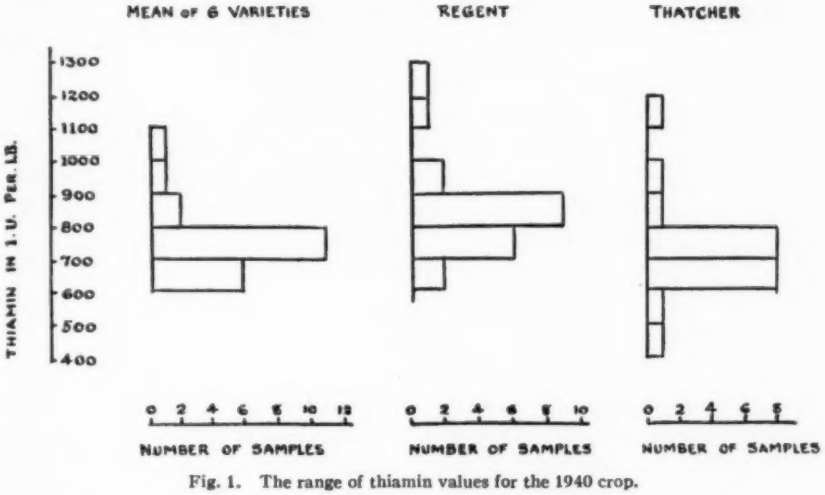

Fig. 1. The range of thiamin values for the 1940 crop.

and for the same varieties for 1940 is shown in Table I. For the 1940 crop, 163 places are represented in the mean figure. It will be observed that the varieties arranged themselves in the same order in 1940 as they did in 1939. Regent, Renown, and Reward gave the highest

thiamin values, and Thatcher, Apex, and Marquis ranked lower. The close similarity between the data for the two years is remarkable.

For the 1940 crop, the study was based on 1,167 original samples and these were composited for certain districts only. A wide range of thiamin values, which included a low of 436 IU per pound for one

TABLE II

MEAN WEIGHT PER BUSHEL, WEIGHT PER 1,000 KERNELS, PROTEIN AND THIAMIN FOR THE SIX VARIETIES FOR EACH DISTRICT AND FOR THE 21 DISTRICT SAMPLES OF EACH OF THE VARIETIES

Crop district means	Samples in composite for each variety	Weight per bushel	Weight per 1,000 kernels	Protein	Thiamin
	no.	*lb*	*g*	*%*	*IU per lb*
MANITOBA					
4	1	59.4	24.9	14.7	768
7	1	59.3	24.2	16.5	979
10	2	62.4	36.4	15.9	804
12	1	58.4	25.3	17.9	1071
SASKATCHEWAN					
1 and 2	4	62.4	31.2	15.4	803
3 and 4	3	62.0	34.6	14.8	607
5	11	62.2	35.5	15.0	617
6	10	62.2	32.9	15.9	722
7	16	61.2	30.5	16.0	722
8	17	61.9	33.1	14.4	657
9	23	62.3	33.3	15.3	753
ALBERTA					
1	1	62.5	32.9	15.6	732
4 and 6	14	61.9	32.0	15.7	763
5	2	61.2	31.8	15.5	757
7 and 8	10	61.7	32.5	15.5	762
9	2	62.9	36.7	12.5	665
10	10	60.8	31.2	15.4	727
11	5	62.7	34.3	13.2	729
12	1	62.3	36.1	14.5	652
13 and 14	15	62.8	35.5	13.8	729
16	4	62.8	34.9	14.0	671
VARIETY MEANS					
Apex	—	61.2	32.1	14.9	695
Marquis	—	61.4	30.9	14.4	660
Regent	—	60.8	36.4	15.1	838
Renown	—	61.8	32.8	15.1	792
Reward	—	63.4	32.2	16.3	784
Thatcher	—	61.4	29.9	14.9	713
Mean for all samples	—	61.7	32.4	15.1	747

sample of Marquis and a high of 1,280 IU per pound for one sample of Regent, was obtained. The bar diagrams (Fig. 1) show a comparison for the mean of the six varieties given in Table I, and for Regent and Thatcher separately. Along with the thiamin data, test weight per bushel, weight per thousand kernels, and protein were determined. This additional information was secured to ascertain if there were any relationships between some of these characters and thiamin as shown by statistical analysis. The mean data for the four characters of the six varieties in all 21 districts are given in Table II. From these values, variance analyses were made and the differences between districts and between varieties were found to be highly significant for each of the four characters. From these variances, standard errors and necessary differences were calculated and these are given in Table III. For thiamin 75 IU per pound are required to indicate

TABLE III

STANDARD ERRORS AND NECESSARY DIFFERENCES CALCULATED FROM THE VARIANCE FOR INTERACTION OF DISTRICTS × VARIETIES

(ND = $2\sqrt{2}$ SE)	Weight per bushel	Weight per 1,000 kernels	Protein	Thiamin
	lb	*g*	*%*	*IU per lb*
SE for single determinations	0.70	1.39	0.53	65.1
SE for mean of district	0.29	0.57	0.22	26.5
SE for mean of variety	0.15	0.30	0.12	14.2
ND for single determination	1.99	3.93	1.49	183.9
ND for mean of district	0.81	1.60	0.61	75.1
ND for mean of variety	0.43	0.86	0.33	40.1

significance between the means of the six varieties for any two districts, and 40 IU per pound between the means for any two varieties for the 21 districts. Similar information is given for weight per bushel, weight per thousand kernels, and protein. These differences are based on the error as determined for the interaction between varieties and districts.

Correlation coefficients calculated from the variance and covariance of the 126 samples representing 21 districts and 6 varieties are presented in Table IV. This arrangement allows for the sorting out of correlations for districts, varieties, and the interaction of varieties and districts. The correlations for district and for total, being significant, show that thiamin is negatively correlated with weight per bushel and kernel weight, and positively correlated with protein. The effect of varieties tends to lower the total correlations as shown in the correlations for varieties. To gain further information on the behaviour of the varieties, simple product moment correlations were calculated for the 21 district samples of each variety. In

Table V it will be noted that all varieties did not behave alike and better relationships for the thiamin combinations were shown for Apex, Renown, and Thatcher.

TABLE IV

CORRELATION COEFFICIENTS FOR WEIGHT PER BUSHEL (w), WEIGHT PER 1,000 KERNELS (k), PROTEIN (p) AND THIAMIN (t) FOR 21 DISTRICT SAMPLES OF EACH OF 6 VARIETIES BASED ON THE VARIANCES AND COVARIANCES OF THE 126 SAMPLES

	DF	wk	wp	wt	kp	kt	pt	5% pt	1% pt
Districts	19	+.92	−.66	−.74	−.62	−.75	+.70	.43	.55
Varieties	4	−.25	+.83	+.15	+.24	+.80	+.60	.81	.92
Districts × varieties	99	+.35	−.03	+.11	−.09	+.18	+.23	.20	.25
Total	124	+.56	−.24	−.39	−.39	−.29	+.61	.17	.23

Kernel weight and bushel weight are positively correlated and each of them is negatively correlated with protein. This was found to be true for the district correlations for all six varieties combined and generally true for each variety taken separately. Further calculations were made to determine the partial and multiple associations of each

TABLE V

CORRELATION COEFFICIENTS FOR 21 DISTRICT SAMPLES OF EACH OF 6 VARIETIES

Variety	DF	wk	wp	wt	kp	kt	pt
Apex	19	+.82	−.68	−.59	−.75	−.77	+.71
Marquis	19	+.78	−.45†	−.57	−.46†	−.43†	+.43†
Regent	19	+.82	−.58	−.26*	−.62	−.52†	+.71
Renown	19	+.85	−.62	−.64	−.63	−.61	+.67
Reward	19	+.93	−.04*	−.49†	−.09*	−.47†	+.30*
Thatcher	19	+.87	−.75	−.76	−.64	−.77	+.71

* Not significant at 5% level. † Not significant at 1% level.

of these characters on thiamin. In Table VI, it will be observed that many of the partial correlations are not significant, although those for Regent are significant or approach significance in 6 out of 9 cases. Thus for Regent, thiamin and protein are correlated when the effects of kernel weight and weight per bushel are eliminated. This follows through for thiamin and protein when kernel weight is eliminated and when bushel weight is eliminated. The district correlations for all varieties combined yielded significant partial correlations for thiamin and kernel weight and thiamin and bushel weight when the effects of protein were eliminated. The multiple correlations are significant in all but five of the various combinations and in these latter cases the correlations approached significance. In general, it

may be stated that the thiamin content was influenced by the same factors that influenced protein, kernel weight, and bushel weight.

Approximately 60 per cent of the variability in the wheat was accounted for by its association with protein, kernel weight, and bushel weight, with protein and kernel weight, with protein and bushel weight, and with kernel weight and bushel weight. On this basis one would expect that when wheat is high in protein and low

TABLE VI

PARTIAL AND MULTIPLE CORRELATIONS FOR 21 DISTRICT SAMPLES OF EACH VARIETY AND FOR 21 DISTRICT TOTALS FOR ALL 6 VARIETIES COMBINED

	Apex	Marquis	Regent	Renown	Reward	Thatcher	District totals	5% pt	1% pt
				PARTIAL CORRELATIONS					
$r_{tp.kw}$	+.34	+.25	+.68	+.43	+.32	+.37	+.43	.60	.69
$r_{tk.pw}$	−.51	+.07	−.50	−.05	+.03	−.46	−.26	.60	.69
$r_{tw.pk}$	+.18	−.33	+.54	−.23	−.24	−.08	−.07	.60	.69
$r_{tp.k}$	+.31	+.29	+.53	+.46	+.29	+.45	+.46	.53	.63
$r_{tk.p}$	−.52	−.29	−.18	−.32	−.46	−.58	−.57	.53	.63
$r_{tp.w}$	+.49	+.24	+.71	+.46	+.32	+.34	+.42	.53	.63
$r_{tw.p}$	−.52	−.46	+.26	−.38	−.51	−.48	−.52	.53	.63
$r_{tk.w}$	−.62	+.04	−.56	−.16	−.07	−.35	−.25	.53	.63
$r_{tw.k}$	+.11	−.40	+.34	−.29	−.19	−.28	−.19	.53	.63
				MULTIPLE CORRELATIONS					
Rt.pkw	+.81	+.54	+.81	+.73	+.57	+.82	+.81	.60	.69
Rt.pk	+.65	+.50	+.69	+.71	+.53	+.82	+.81	.53	.63
Rt.pw	+.49	+.60	+.73	+.73	+.57	+.79	+.79	.53	.63
Rt.kw	+.78	+.57	+.60	+.65	+.50	+.79	+.76	.53	.63

in kernel weight and bushel weight, the thiamin content is higher than when wheat is low in protein and high in kernel and bushel weight.

Jackson and Whiteside (1942) reported that the thiamin in wheat is largely concentrated in that part of the kernel containing the germ, and they and others have shown that commercial bran and shorts are much richer in thiamin than white flour. Therefore, it is logical to assume that plump wheat would be lower in thiamin than less plump wheat, as the relationship of the pericarp and germ to floury endosperm would be greater in the latter case. Since protein was negatively correlated with weight per bushel and weight per thousand kernels, then high thiamin would follow high protein.

Experimental Station Samples

Samples of six varieties grown at the same five stations for two years were analyzed for thiamin content and the data are presented

in Table VII. This arrangement allowed for a further set of statistical analyses which yielded additional information respecting the variability to be found in thiamin values over a period of two years. Variance analyses are presented in Table VIII, from which certain inferences may be made.

TABLE VII

THIAMIN CONTENT FOR SAMPLES OF SIX WHEAT VARIETIES GROWN AT FIVE EXPERIMENTAL STATIONS IN 1940 AND 1941

Varieties	Year	Rosthern	Scott	Melfort	Olds	Edmonton	Mean for varieties
		IU per pound					
Garnet	1940	535	585	517	565	525	545
	1941	695	815	680	640	660	698
	Mean	615	700	599	603	593	622
Marquis	1940	715	665	610	687	640	663
	1941	612	695	640	622	666	647
	Mean	664	680	625	655	653	655
Red Bobs	1940	700	695	631	785	675	697
	1941	811	734	700	666	748	732
	Mean	756	715	666	726	712	715
Regent	1940	806	870	870	735	765	809
	1941	813	883	861	825	798	836
	Mean	810	877	866	780	782	823
Reward	1940	840	840	711	765	712	774
	1941	852	855	700	756	737	780
	Mean	846	848	706	761	725	777
Thatcher	1940	690	611	660	615	630	641
	1941	767	675	668	640	636	677
	Mean	729	643	664	678	633	659
Mean for stations	1940	714	711	667	692	658	688
	1941	758	776	708	692	708	728
	1940, 1941	736	744	687	692	683	708

Significant differences, as shown by the F values exceeding the 5% point or 1% point levels of significance, corroborated the larger series of tests in that some of the varieties could be depended upon to give higher thiamin values than others. This is found to be true regardless of the interaction effect of varieties and stations and of varieties and years. In the first place, as determined through the interaction effects of varieties × stations × years, some varieties were shown to be consistently higher for some stations for some years. In the second place, as determined from the interaction effects of varieties and stations, some varieties were higher in thiamin than the others in these places for both years, and, in the third place, as shown by the interaction effects of varieties and years, some varieties were

higher in thiamin than the others on the average at all stations. The necessary differences are 33.88, 45.97, and 82.96 IU per pound, respectively. Using any of these figures for significance, Regent at 823 IU per pound is significantly higher than Thatcher at 659 IU per pound. The results indicate that varieties differ in thiamin content and that this difference was consistent for different stations

TABLE VIII

STATISTICAL SIGNIFICANCE OF THIAMIN FOR SIX VARIETIES OF WHEAT GROWN AT FIVE STATIONS IN 1940 AND 1941

	Sums of squares	Degrees of freedom	Variance	Standard error
Varieties	305,251.54	5	61,050.31	—
Stations	40,816.17	4	10,204.04	—
Years	24,000.00	1	24,000.00	—
Varieties × stations	52,825.63	20	2,641.28	51.39
Varieties × years	43,020.20	5	8,604.04	92.76
Stations × years	7,157.50	4	1,789.38	42.30
Varieties × stations × years	28,700.30	20	1,435.02	37.88
Total	501,771.34	59	—	—

F VALUES, 5% PT, AND 1% PT

	Varieties × stations × years			Varieties × stations			Varieties × years		
	F value	5% pt	1% pt	F value	5% pt	1% pt	F value	5% pt	1% pt
Between varieties	42.54	2.71	4.10	23.11	2.71	4.10	7.10	5.05	10.97
Between districts	7.11	2.87	4.43	3.86	2.87	4.43	5.70	6.39	15.98
Between years	16.72	4.35	8.10	2.79	6.61	16.26	13.41	7.71	21.20

STANDARD ERRORS AND NECESSARY DIFFERENCES FOR THIAMIN IN IU PER LB

	Varieties × stations × years		Varieties × stations		Varieties × years	
	SE	ND	SE	ND	SE	ND
Mean of a variety	11.98	33.88	16.25	45.97	29.33	82.96
Mean of a station	10.94	30.93	14.84	41.96	Not sig	Not sig
Mean of a year	4.89	13.83	Not sig	Not sig	5.46	15.45

and over a period of two years. This would indicate that the breeding for thiamin content in wheat varieties is a possibility.

In general, it was demonstrated that some stations can be depended upon to give higher thiamin values than others. This was found to be true regardless of interaction effects of varieties × stations × years, and of varieties × stations, but it did not hold true regardless of interaction effects of stations × years only. This would mean that in these years some stations could not be relied upon to be higher in

thiamin for most varieties. More years of test would be necessary to prove or disprove this point.

For years it was shown that significant differences did occur, indicating that the thiamin content will vary from year to year.

Summary

A statistical study was made of the thiamin content of samples of Canadian hard red spring wheat representing leading varieties grown at many locations in Manitoba, Saskatchewan, and in Alberta for the crop years 1940 and 1941. For the 1940 crop a large series of tests on composites were made, representing 1,167 original samples. For this series, thiamin values for 21 different crop districts are given. In the second group samples from five experimental stations for the two years are compared.

For the varieties tested significant differences were shown to exist between varieties. Regent, Renown, and Reward may be expected to be higher in thiamin than Red Bobs, Thatcher, Marquis and Garnet. In a comparison of Regent with Thatcher for 1939 and 1940, representing samples drawn from 158 places in the three Prairie Provinces in 1939 and 163 places in 1940, the former variety averaged 829 and the latter 707 IU per pound. The varieties arranged themselves in the same order for both years. In a comparison of six varieties grown at five stations over a two-year period, the difference between varieties was found to be consistent for different stations and over a period of two years.

Environment was shown to influence the level of thiamin to be expected for wheat from different locations in the same year. These differences were measured by statistical analysis. For the large series of the 1940 crop, 75 IU per pound was found to be necessary to show a difference between the mean of any two districts. On this basis the thiamin values were fairly uniform in that year over the three provinces. In a comparison of samples from five experimental stations for 1940 and 1941, the difference between years was found to be significant.

The thiamin content of wheat appears to be associated with the development of the wheat kernel, as negative correlations were obtained between thiamin and test weight per bushel and between thiamin and weight per 1,000 kernels. Positive correlations were obtained between thiamin and protein. It was calculated that approximately 60% of the variability in thiamin was accounted for by its association with protein, kernel weight, and bushel weight; with protein and kernel weight; with protein and bushel weight; and with kernel weight and bushel weight. The use of any one of these

characters for predicting thiamin content in wheat varieties would appear to be limited.

Acknowledgments

The authors wish to acknowledge the courtesy of the Crop Testing Plan for supplying the samples and the Chemistry Division, Science Service, Dominion Department of Agriculture, for the protein data.

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THE RECOVERY OF THE B VITAMINS IN THE MILLING OF WHEAT

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Wheat is a valuable source of thiamin and niacin, and it supplies significant amounts of riboflavin to the average diet. Hence a knowledge of the routes followed by these vitamins during the milling of wheat is of prime importance. Jackson and Whiteside (1942) have published results on the thiamin concentration to be found in the various streams of a typical flour mill. Thomas, Bina, and Brown (1942) have reported data on the niacin concentration and the percentage of the total niacin of the wheat appearing in the various grades of flour and the offals. Andrews, Boyd, and Terry (1942) reported similar information regarding riboflavin. Andrews, Boyd, and Gortner (1942) have published a graph in which they plotted the percentage of the total thiamin, riboflavin, and niacin to be found in various flours, against the percentage extraction of the flour. The following investigation extends this information by reporting the percentage of the total thiamin, riboflavin, and niacin in all the mill-streams of a typical flour mill.

Experimental

Samples of all the flour streams and mill feeds were supplied by a large Canadian flour mill which was milling a hard western Canadian wheat at the time of collection. A sample of the wheat was also supplied along with data on the percentage of the wheat which appeared in each of the streams. These data made it possible to calculate the

percentage of the vitamins of the wheat in each stream. Assays for thiamin, riboflavin, and niacin were carried out on all the samples.

Thiamin was assayed by the thiochrome method of Hennessy and Cerecedo (1939) as modified later by Hennessy (1941) and the Research Corporation Committee on the Thiochrome Assay.

Riboflavin was assayed by the microbiological method of Snell and Strong (1939). The extraction method was modified to provide complete solution of the riboflavin and to eliminate certain interfering substances peculiar to cereals. The method of extraction used corresponded closely to that published by Strong and Carpenter (1942) as their Procedure 2. An aliquot of the sample under test was weighed out accurately and mixed with 50 ml of 0.1*N* HCl. The mixture, after steaming for about 30 minutes with occasional shaking to break up any lumps, was autoclaved for 30 minutes at 15 pounds of steam pressure. After cooling under the tap to at least room temperature, or even colder, 1 ml of 2.5*M* sodium acetate solution was added, followed by 5 ml of 0.5*N* sodium hydroxide. At this point the pH was between 4.0 and 5.0 and there was a heavy precipitate. This precipitate was removed by centrifuging or filtering or both and appeared to carry down the interfering material. The precipitate was re-extracted by autoclaving with 50 ml of water for 30 minutes at 15 pounds of pressure. It was cooled again, filtered or centrifuged, and the second extract added to the first. The precipitate was washed several times with small amounts of water, the combined washings and extracts adjusted to pH 6.8, and diluted to 200 ml. This extract was assayed by the method of Snell and Strong (1939). A glass electrode titration set-up was used with a distinct improvement in accuracy and convenience over the dye indicator method.

The niacin was assayed by the microbiological method of Snell and Wright (1941) with slight modification. The amount of tryptophan was reduced to half the level recommended and the thiamin, pyridoxine, and calcium pantothenate were doubled. The casein hydrolysate, cystine, guanine, adenine, and uracil were all prepared in a single solution of such concentration that 10 ml was required for 50 ml of the basal medium.

The preparation of the samples for niacin assay involved extraction with dilute acid followed by a mild alkaline hydrolysis. The increase in the apparent niacin of cereal extracts after alkaline hydrolysis has been reported by several investigators (Snell and Wright, 1941; Oser, Melnick, and Siegel, 1941; and Andrews, Boyd, and Gortner, 1942). It is not known as yet whether this extra niacin liberated by alkaline hydrolysis is nutritionally available. As it is very readily liberated (the action of dilute alkali at room temperature for a few minutes

gives almost quantitative hydrolysis), we have assumed that this fraction of the niacin is available, and the results accordingly represent the total niacin in the sample. The extra niacin liberated by the action of the alkali amounted to about 25% of the total value in the whole wheat. It was not determined in the other samples.

A sample was weighed out, mixed with 25 ml of 0.1*N* HCl, autoclaved for 1 hour at 15 pounds of steam pressure, cooled, and neutralized to pH 4.5 with 0.5 ml of 2.5*M* sodium acetate and 2.5 ml of 0.5*N* sodium hydroxide. After diluting the mixture to 50 ml, it was centrifuged, followed by filtration if necessary. A suitable aliquot of this extract (5 to 20 ml) was diluted to about 20 ml with water and made alkaline by the addition of 0.25 ml of 40% NaOH. It was heated to boiling, allowed to cool to room temperature, and the pH adjusted to 6.8 with concentrated HCl, the final adjustment being made with dilute solutions of HCl and NaOH. After dilution to 100 ml, this extract was assayed by the method of Snell and Wright (1941).

Results

Table I gives the amounts of thiamin, riboflavin, and niacin found in the various mill feeds and flours constituting all of the original wheat. The total recoveries should be 100% and the variation from

TABLE I

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE MILL FEEDS AND FLOUR COMPOSITES COMPOSING 100% OF THE WHEAT

Mill stream	Percent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Wheat	100	4.52	1.24	56.5	100	100	100	
Feed middlings	2.64	20.8	3.02	95.0	12.1	6.45	4.45	—
Shorts	11.7	15.15	2.48	140.0	39.8	23.4	29.0	—
Bran	11.7	6.98	2.38	232.0	18.1	22.3	48.0	—
Germ	0.27	21.3	4.53	68.0	1.25	0.97	0.32	—
First patent flour	37.0	0.81	0.67	10.3	6.63	20.0	6.75	0.36
First baker's flour	25.8	1.21	0.54	17.6	6.9	11.2	8.0	0.48
Second baker's flour	7.4	2.44	0.76	25.3	4.0	4.55	3.32	0.67
Low-grade flour	3.7	8.15	1.05	41.5	6.66	3.13	2.72	1.16
	100.21	—	—	—	95.44	92.00	102.56	—

this amount is due to the experimental error of the various assay methods. The highest concentration of thiamin was found in the germ and feed middlings fractions, while the bran was the lowest of

the mill feeds, even lower than the low-grade flour. There was a large variation in the concentration of thiamin in the different fractions of the wheat. The thiamin in the germ was 46 times as concentrated as it was in the lowest stream (the first middlings flour, Table II). The shorts contained more of the total thiamin in the wheat than any other single fraction, while approximately one-quarter of the thiamin appeared in all the flour combined.

TABLE II

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE FIRST PATENT FLOUR

Mill stream	Percent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Second midds flour	9.6	0.72	0.59	8.7	1.53	4.58	1.47	.37
First midds flour	9.6	0.46	0.55	9.9	0.98	4.25	1.68	.35
Third midds flour	7.4	0.99	0.54	10.5	1.62	3.22	1.37	.42
Fine sizings flour	0.925	1.04	0.55	11.0	0.21	0.406	0.18	.47
Third midds rebolt	2.95	1.23	0.55	13.0	0.80	1.31	0.68	.42
Second midds rebolt	3.7	1.27	0.53	11.6	1.04	1.58	0.76	.43
Fourth midds rebolt	2.77	1.11	0.53	11.0	0.68	1.18	0.54	.44
Second tails flour	0.37	3.57	0.58	33.0	0.29	0.173	0.26	1.06

The riboflavin was much more evenly distributed throughout the kernel than the thiamin. In the germ fraction, where it was most concentrated, it was only 8.5 times the concentration in the lowest stream. Again the shorts contained more of the total riboflavin than any single fraction, but the total combined flours contained 40% of the riboflavin as compared to 25% of the thiamin.

The variation of the niacin concentration in the different fractions was not quite so great as that of the thiamin, but considerably more than that of the riboflavin. The bran stream contained the highest concentration. This was 26 times the lowest concentration, which was found in the second middlings flour. The outstanding feature is the fact that the niacin was largely concentrated in the bran. This fraction contained nearly half the total niacin of the wheat. The germ stream contained a concentration of niacin that was not much greater than that of the original whole wheat. This germ fraction contained a considerable proportion of bran. A sample of pure germ, entirely free of bran, was separated out by hand and assayed for niacin. It contained 42 μg of niacin per gram, or about the same as the low grade flour. The combined flour streams contained a total of approximately one-fifth of the niacin. The concentration of thiamin

in the second baker's flour was approximately three times that in the first patent flour. The concentration of niacin increased at a similar rate, the second baker's flour having about 2½ times the concentration of niacin of the first patent flour. The similarity vanishes as we progress to the low-grade flour. This had a concentration of thiamin equal to nearly 10 times that of the first patent flour, while the niacin concentration of the low-grade flour was 4 times that of the first patent. This finding agrees with that of Andrews, Boyd, and Gortner (1942),

TABLE III

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE FIRST BAKER'S FLOUR

Mill stream	Percent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Fourth midds scalp	0.74	1.11	0.54	16.0	0.18	0.322	0.21	0.49
Coarse sizings	0.55	1.29	0.54	14.0	0.155	0.24	0.135	.48
Third sizings flour	2.22	0.96	0.55	16.0	0.47	0.985	0.63	.47
Fifth midds flour	5.16	1.26	0.60	15.0	1.44	2.50	1.37	.44
Fourth midds flour	5.91	1.05	0.52	16.0	1.37	2.48	1.68	.46
Fifth midds rebolt	2.96	1.62	0.55	10.5	1.06	1.31	0.55	.36
Sixth midds flour	4.44	1.93	0.58	13.5	1.89	2.07	1.06	.52
Fine tailings reel	0.74	1.44	0.55	14.5	0.235	0.328	0.19	.55
Fine tailings bolter	0.925	1.67	0.62	15.0	0.34	0.46	0.245	.55
Suction flour	0.925	1.32	0.70	15.0	0.28	0.52	0.245	.64
First break flour	0.37	0.55	0.66	22.5	0.045	0.197	0.145	.59
Second break flour	0.37	0.51	0.66	19.8	0.04	0.197	0.13	.49
Third break flour	0.37	0.77	0.60	16.4	0.065	0.179	0.105	.52
Fourth break flour	0.185	1.83	0.77	19.3	0.075	0.115	0.065	.75

who plotted the % recovery of thiamin, riboflavin, and niacin in flours of various extractions against the percent extraction of the flours. There was a sharp increase in the thiamin between 70% and 80% extraction, while the rate of increase in niacin remained more gradual until the extraction reached 85% or more, and a considerable percentage of bran was appearing in the flour.

Tables II, III, IV, and V record the amounts of thiamin, riboflavin, and niacin in the individual flour streams composited in the first patent, first baker's, second baker's, and low-grade flours, respectively. These values are of general interest to millers and a study of their relations to each other yields further information. The break flours were relatively low in thiamin but high in niacin. There was an average ratio of the percentage recovery of niacin to thiamin of 1.99 in the break flours and of 0.88 in the middlings flours. This characteristic of high ratio of niacin to thiamin in the break flours can be

TABLE IV

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE SECOND BAKER'S FLOUR

Mill stream	Percent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Fourth break rebolt	0.555	1.51	0.55	29.0	0.185	0.246	0.285	0.68
Seventh midds flour	1.48	3.7	0.6	27.6	1.21	0.715	0.725	.82
Sixth midds rebolt	2.22	3.01	0.75	18.5	1.475	1.34	0.73	.57
Third tailings flour	0.555	2.39	0.56	19.2	0.29	0.25	0.19	.67
Germ flour	0.185	1.82	0.56	16.0	0.073	0.084	0.05	.52
First tailings flour	0.555	3.27	0.90	28.0	0.40	0.402	0.275	.77
Break sifts reel	1.85	0.93	0.74	17.5	0.38	1.1	0.575	.47

TABLE V

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE LOW-GRADE FLOUR

Mill stream	Percent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Second low grade	0.925	13.45	0.92	40.0	2.76	0.685	0.655	1.50
Fifth break flour	0.555	3.15	1.01	40.0	0.385	0.452	0.395	1.27
First low grade	1.11	13.4	0.76	35.0	3.3	0.68	0.69	1.08
Bran and shorts duster	1.11	3.60	1.11	52.0	0.885	0.995	1.02	1.35

explained by the inclusion of some bran dust in these streams, since the bran would add considerable niacin but little thiamin.

Table VI gives the concentrations of the vitamins in the streams related to the production of Vi-Bim. Vi-Bim is a special high-thiamin product that is used for addition to white flour to raise its thiamin

TABLE VI

THE B VITAMINS IN "VI-BIM"[1] AND ITS COMPONENT STREAMS

Stream	Thiamin	Riboflavin	Niacin
	μg/g	μg/g	μg/g
Vi-Bim	23.7	3.01	110.0
Overs, Vi-Bim sifters	25.3	3.17	114.0
First scalp tailings	25.0	3.06	101.0
Tail red dog reel	15.1	3.03	110.0
Shorts from duster	22.1	2.97	100.0
Shorts from graders	11.5	3.11	188.0

[1] Vi-Bim is a special high-thiamin-content concentrate used for addition to white flour to increase the thiamin content to the level required for "Canada Approved" flour. It is made by regrinding and sifting the component streams.

content to the level required for "White Flour, Canada Approved." This level is set at 2.4 to 2.65 μg per gram. Vi-Bim is produced by further grinding and sifting of the first scalp tailings, the tailings from the red dog reels, and the shorts from the dusters and the graders. The concentration of riboflavin is nearly the same in the various streams. The concentration of thiamin in the shorts from the graders is the lowest, whereas the concentration of niacin in this stream is the highest. The addition of sufficient Vi-Bim to the first baker's flour to increase the thiamin to 2.5 μg per gram, an increase of 106%, will raise the niacin content of the flour by 34% and the riboflavin content by 30%.

Conclusions

The fact that nearly half the total niacin of the wheat appears in the bran stream leads to the conclusion that the niacin of the wheat kernel must be largely contained in the branny layers. The data also show that the site of the highest concentration of niacin in the wheat kernel is quite different from the site of the thiamin. The concentration of niacin in each of the four offal streams varies inversely as the concentration of thiamin. That is, the bran stream is highest in niacin and lowest in thiamin, while the germ is the reverse. The shorts and feed middlings lie between in each case but the inverse variation holds with them also.

The exact site of the maximum thiamin concentration in the wheat kernel cannot be placed on the basis of this evidence. We can say that it is not in the bran layers. It appears to be in a lighter-colored portion of the wheat, because fractions containing a high concentration of thiamin may be only a light gray in color. Vi-Bim conforms to this description, while the low-grade flour itself is higher in thiamin than the bran and is very much lighter in color. A previous report by Jackson and Whiteside (1942) placed 80% of the thiamin in that third of the wheat kernel containing the germ.

Some dissections carried out on a sample of soft Ontario winter wheat place the thiamin of the wheat kernel more accurately. A soft wheat was chosen as it was easier to dissect than a hard wheat. A sample of large, well filled kernels was carefully selected and a portion assayed directly for thiamin. A second portion was weighed and the easily removable part of the germ (consisting largely of embryo) was dissected out and discarded. The wheat remaining was weighed and found to constitute 98% of the whole wheat. It was then assayed for thiamin.

A third sample of wheat was weighed out and the entire germ, comprising both the embryo and the scutellum, was carefully dissected

out down to the floury endosperm cells and discarded. Care was taken to remove as few of the epidermal and aleuron cells adjacent to the germ as possible. The part of the wheat remaining was found to constitute 93.2% of the whole wheat. It was also assayed for thiamin.

Finally a fourth sample of wheat was weighed out and about a quarter of each kernel, comprising the entire germ and all adjacent bran and endosperm cells, was cut off and discarded. The remainder of the wheat, constituting 73% of the whole wheat, was assayed for thiamin.

The following results were obtained. The whole wheat contained 3.56 μg of thiamin per gram. The wheat with the embryo removed contained 3.2 μg per gram of the sample assayed, or 3.14 μg per gram of the original wheat. The wheat with the embryo and scutellum removed contained 1.11 μg per gram of the sample, or 1.035 μg per gram of the original wheat; and the wheat with the entire germ end removed contained 1.11 μg per gram of the sample, or 0.81 μg per gram of the whole wheat. It was calculated from this that the embryo contained 21 μg of thiamin per gram (corresponding closely to that reported for the germ in Table I), and carried 11.8% of the thiamin of the wheat. The scutellum was found to contain 44 μg of thiamin per gram and carried 59% of the total thiamin in the wheat. The bran and endosperm cells adjacent to the germ were found to contain only 1.12 μg of thiamin per gram. The entire wheat kernel other than the embryo and scutellum (93.2% of the wheat) contained only 30% of the thiamin. This bears out in general the finding recently reported in England by Hinton (1942). He found the scutellum to contain about 40 μg of thiamin per gram but reports a value of only 4 μg per gram of the embryo. The divergence in values found for the embryo probably depends on the accuracy of the dissection. In this case the part dissected out as embryo comprised 2% of the wheat and may have carried some of the scutellum with it. Hinton found the embryo to comprise 1.2% of the wheat.

The riboflavin appears to be more evenly distributed throughout the kernel, the floury endosperm containing a fair proportion of the total riboflavin in the wheat while all the offal streams combined contain only a little more than half of this vitamin.

Increase of the thiamin in flour by variation of the milling process is not necessarily accompanied by a like increase of the riboflavin and niacin. The riboflavin would not be materially increased because there is no single part of the wheat kernel which contains most of the riboflavin and which could be incorporated in the flour by a suitable milling procedure. The niacin cannot be materially increased, be-

cause it is contained largely in the outer bran layers of the kernel and these must be discarded if the flour is to be generally acceptable.

Summary

Samples of all the flour and feed streams of a large Canadian flour mill were assayed for thiamin, riboflavin, and niacin. The proportion of the total vitamin of the wheat that appeared in each of the streams and in the flour composites was calculated. The niacin appeared largely in the outer bran layer of the wheat kernel. Dissection experiments showed the thiamin to be largely concentrated in the scutellum portion of the wheat germ. The riboflavin was found to be more evenly distributed than the thiamin or niacin.

Acknowledgment

The authors wish to express their gratitude to Mr. N. L. Gregory of the Maple Leaf Milling Company, Ltd., for his kind assistance in supplying the samples for analysis and data relating to the percentage of the wheat appearing in each stream.

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A STUDY OF SOME OF THE VITAMIN B-COMPLEX FACTORS IN RICE AND ITS MILLED PRODUCTS

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(Received for publication April 27, 1943)

In the past few years, efforts to incorporate the vitamin-rich by-products of rice milling into the human diet have aroused a great deal of interest. The present study was undertaken in the fall of 1941 in an attempt to meet the increasing need for a comprehensive survey of the B-complex vitamin content of rice and its milled products. The survey has included thiamine, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, biotin, and inositol. In the present report, data on the first four of the vitamins named have been summarized.

While to the authors' knowledge no such comprehensive study has been made of rice products, some analyses with regard to several of the vitamins have been made on various kinds of rice samples. Nelson and Palmer (1942) investigated the thiamine, riboflavin, nicotinic acid, and pantothenic acid content of wild rice, and Kik (1943) studied the thiamine content of milled fractions and varieties of rice. Innumerable studies have been made on the thiamine content of Indian rices. Data on pyridoxine, biotin, and inositol for a group of samples such as analyzed here have not been available.

Experimental

During the height of the 1942–43 milling season, one hundred samples were collected from 13 Louisiana mills and one Arkansas mill. The collection of samples from many mills rather than one mill was held desirable in order to obtain data representative of milled samples in general. Samples of rice were collected during the milling season of 1941–42, but the results of analyses were used for comparison only. The samples were divided into two main groups: (1) samples of brown rice of six outstanding Louisiana varieties (Blue Rose, Fortuna, Early Prolific, Nira, Rexora, and American Pearl) collected for the purpose of studying differences among varieties, and (2) samples of milled fractions of three typical varieties (Blue Rose, Early Prolific, and Fortuna) for the purpose of studying differences in vitamin content of the different milled fractions.

These milled fractions consist of the products obtained through the different operations in the milling process, as more and more of the outer coats of the rice grain are removed and as the rice progresses

from the original rough state to the finished product. In Louisiana, the fractions usually consist of: (1) brown rice, (2) first-break rice, (3) second-break rice, (4) brushed rice, and (5) finished rice. The by-products of the milling process are rice polish and rice bran, which may also be considered milled fractions and consist of the removed outer coats of the grain. The samples were analyzed by the following procedures:

Thiamine: An adaptation of the Hennessy and Cerecedo (1939) method for the determination of thiamine, as prepared by the Research Corporation Committee on the Thiochrome Method, was used. This method was followed in detail, except that the isobutanol was reclaimed by agitation with activated charcoal and filtration according to the method of Pader (1943).

Pantothenic acid: The microbiological method of Pennington, Snell, and Williams (1940) was followed. It was found that autoclaving the samples with 75 ml of water at 15 pounds of pressure for 15 minutes and adjusting to an appropriate volume yielded the best results.

Nicotinic acid: The microbiological method of Snell and Wright (1941) was employed. Samples of rice polish and rice bran were hydrolyzed in 0.1*N* alkali at 15 pounds of pressure for 15 minutes, cooled, neutralized, and made up to an appropriate volume. Brown rice and milled fractions were autoclaved in water solution and subsequently treated like the bran and polish.

Pyridoxine: The Bird, Vandenbelt, and Emmett adaptation of the Scudi colorimetric method (1941) was used. The samples were extracted as for thiamine. It was found that more consistent readings were obtained (1) by taking readings 40 minutes after the dye solution had been added and (2) by drying the butanol phase with anhydrous sodium sulfate before making the readings, in accord with Scudi (1941).

Conclusions

With reference to the influence of variety and milling, two conclusions were drawn.

TABLE I

COMPARISON OF THE VITAMIN CONTENT OF SEVERAL VARIETIES OF BROWN RICE[1]

Variety	Vitamin content			
	Thiamine	Nicotinic acid	Pantothenic acid	Pyridoxine
		μg per gram of dry weight		
Blue Rose	4.0	49.3	16.5	9.4
Early Prolific	4.2	45.5	15.9	10.3
Nira	5.2	60.4	17.3	10.2
Rexora	4.3	41.8	15.4	10.7
American Pearl	3.6	39.1	14.6	10.4
Fortuna	4.4	46.9	18.6	11.2
Composite average	4.3	46.2	16.4	10.3

[1] All figures represent averages of a number of samples.

No significant differences in the vitamin content of the different varieties were demonstrated. Some slight differences, however, were apparent, if one compared the individual varieties with average values. The long-grain rices, such as Nira and Fortuna, were highest in vitamin

content. The Rexora and Blue Rose occupied an intermediate position, and the shorter-grained varieties, Early Prolific and American Pearl, were lowest. This finding seemed reasonable, since in general the surface area per volume of the grain and hence the area of the bran layers is greater in the longer-grain varieties. No sharp distinctions were noticeable, however, and the differences were small (Table I).

With regard to the effect of milling, the vitamin content was found to decrease as the rice progressed through the successive states of milling (Table II). This finding was in accord with the generally

TABLE II

COMPARISON OF THE VITAMIN CONTENT OF THE MILLED FRACTIONS OF SEVERAL VARIETIES OF RICE[1]

Vitamin	Variety	Milled fractions						
		Brown rice	1st break	2nd break	Brushed	Finished	Bran	Polish
		μg per gram of dry weight						
Thiamine	Blue Rose	4.0	1.2	1.2	0.9	0.8	26.9	25.9
	Fortuna	4.4	1.6	0.9	0.5	0.6	26.6	28.5
	Early Prolific	4.2	2.3	1.4	1.0	1.2	30.1	15.6
	Average[2]	4.2	1.7	1.2	0.8	0.8	27.9	23.3
Nicotinic acid	Blue Rose	49.3	23.7	18.2	13.7	12.3	403.6	403.1
	Fortuna	46.9	20.4	17.0	9.3	9.6	487.4	429.8
	Early Prolific	45.5	33.2	29.9	14.0	16.1	334.8	321.2
	Average[2]	47.2	25.8	21.7	12.3	12.7	408.6	384.7
Pantothenic acid	Blue Rose	16.5	8.1	7.5	7.0	6.5	65.9	94.9
	Fortuna	18.6	8.6	7.7	6.1	6.3	81.3	110.6
	Early Prolific	15.9	10.5	8.0	9.6	6.5	66.8	72.0
	Average[2]	17.0	9.1	7.7	7.6	6.4	71.3	92.5
Pyridoxine	Blue Rose	9.4	4.3	2.6	2.6	2.0	23.8	29.1
	Fortuna	11.2	7.6	4.9	5.2	5.3	33.8	31.2
	Early Prolific	10.3	8.8	7.7	7.3	6.2	38.6	32.1
	Average[2]	10.3	6.9	5.1	5.1	4.5	32.1	30.8

[1] All figures represent averages of a number of samples.
[2] Composite average.

accepted fact that these members of the B-complex were located in the bran coats and germ of the rice grain. The greatest drop in vitamin content came with the first break, hence demonstrating the inadequacy of the oft-proposed idea of marketing an under-milled rice as a high source of B vitamins.

The values obtained for thiamine, nicotinic acid, and pantothenic acid on the whole were in good agreement with those obtained by other workers. The values for the pyridoxine content of finished or polished

rice were in agreement with the findings of Swaminathan (1940), but the values for rice polish were much higher than those of the latter. Differences in methods of milling, amounts of broken rice and hulls in the polish, etc., might have been responsible for the disagreement.

As one may readily see from the data, rice polish and rice bran are excellent sources of thiamine, nicotinic acid, pantothenic acid and pyridoxine. The brown rice, though lower, is yet a good source.

Acknowledgment

The authors gratefully acknowledge the assistance of Dr. Horace J. Davis, Dr. T. D. Kroner, and Mr. Sam Henderson in the preliminary work.

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RIBOFLAVIN IN PRODUCTS OF COMMERCIAL RICE MILLING AND THIAMIN AND RIBOFLAVIN IN RICE VARIETIES [1]

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(Received for publication December 15, 1942)

Reports have been made on the riboflavin content of wheat and corn (Conner and Straub, 1941; Andrews, Boyd, and Terry, 1941); rye and its milled products (Ihde and Schuette, 1941); and wild rice

[1] Research paper No. 771, Journal Series, University of Arkansas, published with the approval of the Director of the Arkansas Agricultural Experiment station. Aided by a grant from the Williams-Waterman Fund of the Research Corporation.

(Nelson and Palmer, 1942). No such data have been published on cultivated rice.

This investigation deals with the riboflavin content of products of commercial rice milling. In addition, a few samples of parboiled and undermilled rice have been tested. Also included are data on the thiamin and riboflavin contents of different rice varieties obtained in four different states—Arkansas, Louisiana, Texas, and California.

The essential steps in milling, description of the main products and byproducts, the obtaining of samples, and the method for the analysis of thiamin were described in a previous publication (Kik, 1942).

Experimental

The samples were analyzed for riboflavin by a fluorometric method (Conner and Straub, 1941). The method, as used in the laboratory, is briefly stated as follows: Samples of 5–10 g (depending on vitamin content) are transferred to 200-ml amber-colored Pyrex Erlenmeyer flasks, suspended in 75 ml of 0.1*N* H_2SO_4, autoclaved in a pressure cooker for 20 minutes at 15 pounds pressure, cooled, adjusted to pH 4.0 to 4.5 with 2*N* sodium acetate solution with brom cresol green as an outside indicator. In order to obtain a clear filtrate, 125 mg of takadiastase is added, mixed and incubated at 50°C for 1 hour. After incubation the mixture is cooled, made up to 100 ml, and filtered in the dark. Thirty-milliliter aliquots of the clear extracts are used for the riboflavin determination, with florosil used as a means of absorption and pyridine-acetic acid as an eluting agent. A blank determination, containing reagents and takadiastase, was run simultaneously with the samples.

Results

Table I shows that rough rice contained an average of 0.67 μg per gram of riboflavin, while brown rice contained 0.53 μg, or less than rough rice. During the milling process considerable losses of riboflavin took place. Whole rice of Supreme Blue Rose variety (mill lot 551) had a riboflavin content of 0.47 μg per gram, which was reduced in milling to 0.28 μg per gram of head rice, which is sold for human consumption. There was a loss of 40.4%. In the second mill lot (606) of the same variety, 55.1% was lost. The other varieties and mill lots showed similar losses. Early Prolific lost 45.6% in mill lot 569 and 47.0% in mill lot 663. Fortuna lost 59.0% and Lady Wright lost 66.6%, while the loss in Improved Blue Rose amounted to 35.4%.

The average loss of the seven mill lots was 50.0% of the riboflavin originally present in the whole brown rice. The average riboflavin content of the head rice was 0.26 μg per gram, and of the second head,

0.25 μg per gram. Screenings and brewer's rice had an average riboflavin content of 0.34 and 0.36 μg per gram, respectively.

Of the byproducts, rice hulls contained an average of 0.76 μg per gram. Rice bran and rice polish were found to be better sources of riboflavin than the main products. Rice bran (first break) contained an average of 2.68 μg per gram. Second-break bran contained less

TABLE I

RIBOFLAVIN CONTENT OF PRODUCTS OF COMMERCIAL RICE MILLING [1]

Products	Variety[2] and mill lot						
	Supreme Blue Rose		Early Prolific		Fortuna	Lady Wright	Improved[3] Blue Rose
	551	606	569	663	635	768	778
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
Paddy or rough rice	0.58	0.57	0.67	0.57	0.67	0.69	0.93
From milling or bleaching process:							
Whole brown rice	0.47	0.49	0.57	0.49	0.66	0.57	0.48
First break huller rice	0.22	0.20	0.38	0.26	0.26	0.28	0.28
Second break huller rice	0.25	0.20	0.34	0.25	0.24	0.33	0.28
Pearling cone rice	0.29	0.20	0.39	0.25	—[4]	—[4]	0.34
Brush rice	0.19	0.20	0.38	0.31	0.24	0.28	0.12
Finished, clean, polished rice:							
Head rice	0.28	0.22	0.31	0.26	0.27	0.19	0.31
Second head rice	0.22	0.26	0.38	0.24	0.26	0.22	0.20
Screenings	0.38	0.25	0.41	0.30	0.32	0.38	0.31
Brewers rice	0.40	0.32	0.49	0.38	0.40	0.25	0.38
Rice byproducts:							
Hulls	0.66	0.82	0.95	0.64	0.62	0.93	0.71
First break bran	3.33	2.63	2.66	2.05	3.30	2.80	2.00
Second break bran	1.37	1.47	1.84	1.67	2.20	2.02	1.80
Pearling cone polish	1.52	1.87	1.59	1.52	—[4]	—[4]	1.62
Brush polish	1.26	1.29	1.14	1.44	1.44	1.60	1.18

[1] Obtained from one mill, through the courtesy of the Walton Mill, Inc., Stuttgart, Arkansas.
[2] All varieties are from fields which were not fertilized and were irrigated by well water.
[3] Grown in Louisiana.
[4] Pearling cones are not used in long grain varieties.

(1.77 μg/g). Rice polish (pearling cone) had 1.62 μg/g, and brush polish 1.34 μg/g. Both brush and pearling cone polish contained less riboflavin than bran, which suggests that most of the riboflavin is present in the outer layers of the rice kernel.

The results of assays of a few samples of parboiled and undermilled rice are shown in Table II, and indicate that the riboflavin content in milled parboiled rice is somewhat higher than in milled nonparboiled rice. A sample of milled parboiled rice of the Nira variety contained

0.47 μg per gram and a sample of milled nonparboiled rice of the same variety and mill lot contained 0.30 μg per gram. A sample of milled Indian parboiled rice contained 0.30 μg per gram, and a sample of milled parboiled California rice (Caloro variety) had 0.33 μg per gram, compared with an average of 0.26 μg for ordinary milled rice (Table I). However, more samples of parboiled rice must be analyzed in order to establish this point completely, and the influence of parboiling on riboflavin content deserves further investigation.

TABLE II

RIBOFLAVIN CONTENT OF MILLED PARBOILED AND MILLED NONPARBOILED RICE, AND MILLED AND UNDERMILLED RICE

Variety	Treatment	μg/g
Nira[1]	Milled, parboiled	0.47
Caloro[2]	Milled, parboiled	0.33
Indian[3]	Milled, parboiled	0.30
Nira[1]	Milled, nonparboiled	0.30
Lady Wright[3]	Undermilled	0.28
Lady Wright[3]	Milled	0.19
Supreme Blue Rose[3]	Undermilled	0.32
Supreme Blue Rose[3]	Milled	0.25

[1] Obtained through the courtesy of Dr. C. R. Adair, Associate Agronomist, U. S. Department of Agriculture, Bureau of Plant Industry, Rice Branch Experiment Station, Stuttgart, Arkansas.
[2] Obtained through the courtesy of the Rice Growers Association of California, Sacramento, California.
[3] Obtained through the courtesy of the Arkansas Rice Growers Cooperative Association, Stuttgart, Arkansas.

The riboflavin content of undermilled rice is higher than that of ordinary milled rice, which is evident from Table II. An undermilled sample of Supreme Blue Rose contained 0.32 μg per gram, compared with 0.25 μg for the sample of ordinary milled rice.

Samples of rough rice or paddy, obtained from the state agricultural experiment stations of the main rice-producing states, Arkansas, Louisiana, Texas, and California, were analyzed for their thiamin and riboflavin content. These samples were from the following 18 varieties: Acadia, Caloro, Arkrose, Improved Blue Rose, Prelude, Lady Wright, Zenith, Arkansas X Fortuna, Early Prolific, Nira, Calady, Calady 40, Colusa, Supreme Blue Rose, Rexora, Fortuna, Japan, and Blue Rose. Ten varieties were obtained from Arkansas, 10 from Louisiana, 7 from Texas, and 5 from California. One variety (Early Prolific) was grown in all four states; Lady Wright, Nira, and Zenith were cultivated in three states; the remaining varieties in one or two states. The results of these assays are given in Table III.

It is evident from these data that the thiamin content of rough rice is considerably higher than that of riboflavin; the average thiamin content of all varieties is 3.85 μg per gram compared with 0.59 μg of

TABLE III

THIAMIN AND RIBOFLAVIN CONTENT OF PADDY OR ROUGH RICE (HARVEST, 1941) FROM RICE VARIETIES FROM FOUR DIFFERENT STATES

Variety and location		Thiamin	Riboflavin
		μg/g	μg/g
Early Prolific	SA[1]	4.20	0.64
	CL[2]	4.40	0.54
	BT[3]	4.00	0.61
	BC[4]	3.16	0.50
Caloro	SA	4.00	0.54
	CL	4.00	0.76
	BC	2.87	0.66
Arkrose	SA	4.20	0.60
	CL	4.00	0.63
Acadia	SA	3.80	0.60
	CL	4.09	0.83
Prelude	SA	3.31	0.64
	CL	3.75	0.66
Supreme Blue Rose	SA	3.55	0.57
Improved Blue Rose	CL	4.15	0.59
Blue Rose	BT	5.06	0.51
	BT	3.46	0.54
	BT	3.58	0.52
Lady Wright	SA	4.55	0.58
	CL	4.06	0.52
	BC	2.75	0.66
Nira	SA	4.63	0.62
	CL	4.74	0.60
	BT	3.30	0.47
	BT	5.05	0.43
Arkansas X Fortuna	SA	4.63	0.62
	CL	4.13	0.63
Zenith	SA	3.75	0.47
	CL	3.63	0.64
	BT	4.00	0.52
	BT	3.52	0.68
Japan	BT	3.46	0.74
	BT	3.07	0.59
Rexora	BT	3.70	0.61
	BT	4.00	0.45
Fortuna	BT	3.58	0.44
	BT	4.26	0.45
Calady	BC	2.63	0.73
Calady 40	BC	3.34	0.65
Colusa	BC	3.86	0.51

[1] From Rice Branch Experiment Station, Stuttgart, Arkansas.
[2] From Rice Branch Experiment Station, Crowley, Louisiana.
[3] From Rice Grading Service, American Rice Growers' Cooperative Association, Beaumont, Texas.
[4] From Rice Experiment Station, Biggs, California.

riboflavin. The highest thiamin content (5.06) was found in a sample of Blue Rose grown in Texas, and the lowest (2.63) was from a sample of Calady from California. Riboflavin ranged from 0.43 to 0.83 μg per gram. These data indicate that rice varieties differ in thiamin and riboflavin content.

The possible effect of locality on the thiamin and riboflavin content can be observed from the data of samples of the same varieties grown in different states. Early Prolific from four states showed similar thiamin content in Arkansas (4.20), Louisiana (4.40), and Texas (4.00). The same variety grown in California had a thiamin content of 3.16 μg per gram. Similar observations were made on thiamin and riboflavin content of the varieties Caloro, Lady Wright, and Nira.

Summary

The riboflavin content of products of commercial rice milling has been determined.

An average of 50% of the riboflavin was lost during the milling process.

Of the finished, clean products, the end product head rice (sold for human consumption) contained an average of 0.26 μg and second head 0.25 μg per gram of dry matter. Screenings and brewer's rice contained 0.34 and 0.36 μg riboflavin, respectively. These differences are too small to be of any significance.

Of the byproducts, hulls contained 0.76 μg, bran from 1.37 to 3.33 μg per gram, and rice polish 1.14 to 1.87 μg per gram.

Three different samples of milled parboiled rice (prepared in three different localities) contained 0.47, 0.33, and 0.30 μg of riboflavin per gram of dry material.

Two different samples of undermilled rice contained 0.28 μg and 0.32 μg, compared with 0.19 μg and 0.25 μg per gram in the milled rice.

The average thiamin content of all varieties was 3.85 μg, compared with 0.59 μg of riboflavin.

The thiamin and riboflavin content of rice differed with variety and locality.

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THE INFLUENCE OF PROCESSING ON THE THIAMIN, RIBOFLAVIN, AND NIACIN CONTENT OF RICE[1]

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(Received for publication January 8, 1943)

In previous communications (Kik, 1943; Kik and Van Landingham, 1943) studies were reported in which a loss of 80% in thiamin and of 50% in riboflavin in rice, as a result of the milling, were recorded. To prevent such losses, a new rice milling procedure should be developed or the same process of milling should be applied to a different type of rice.

A survey of the literature revealed that an English firm has developed and patented a process (H.R. Rice Conversion, 1940) that produces a new type of rice. The firm claims that its finished milled product, the so-called converted rice, has a thiamin content much higher than that of ordinary milled white rice. The vitamin was determined by the rat bradycardia method.

Studies on methods for the retention of thiamin in rice have been conducted in the Philippine Islands (Diy, 1941), where a qualitative histological procedure for the determination of the thiamin content was used. From these investigations the author concluded that husked, unpolished rice retains a large proportion of the thiamin content after it has been steamed under reduced pressure at a temperature ranging from 50° to 60°C, dried, and milled.

This paper reports studies on the thiamin, riboflavin, and niacin content of rice processed according to the principles of the H. R. Rice Conversion method.

Sixty-gram samples of rough rice were cleaned with cold distilled water and the wet grains were successively exposed to vacuum, hot water under pressure, and steam. They were finally dried and milled. In order to insure the same degree of milling, treated and untreated samples were milled for the same length of time. The milled samples

[1] Research paper No. 772, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station. Aided by a grant from the Williams-Waterman Fund of the Research Corporation.

TABLE I

INFLUENCE OF PROCESSING ON THIAMIN, RIBOFLAVIN, AND NIACIN IN RICE

Sample	Thiamin					Riboflavin					Niacin				
	Non-milled	Converted, milled	Unconverted, milled	Retention method		Non-milled	Converted, milled	Unconverted, milled	Retention method		Non-milled	Converted, milled	Unconverted, milled	Retention method	
				New	Old				New	Old				New	Old
	μg/g	μg/g	μg/g	%	%	μg/g	μg/g	μg/g	%	%	μg/g	μg/g	μg/g	%	%
7 [1]	3.70	2.35	0.82	63.5	22.2	0.52	0.40	0.28	76.8	52.6	54.1	43.5	25.0	80.0	46.2
18 [1]	3.70	1.90	.63	51.3	17.0	.52	.40	.31	78.3	60.0	54.1	32.3	14.0	60.0	25.8
23 [1]	3.70	2.85	.55	77.0	15.0	.52	.39	.31	75.8	60.0	54.1	47.8	14.7	88.3	27.1
C [2]	3.46	2.50	.50	72.2	14.1	.53	.28	.19	52.0	35.3	52.3	31.2	15.7	60.0	30.0
D [2]	3.62	2.70	.40	74.5	11.0	.42	.29	.15	70.0	36.1	53.0	40.0	21.0	75.4	39.6
100 [2]	4.00	3.10	.65	77.5	16.2	.52	.40	.28	78.3	54.5	55.0	44.0	18.0	87.2	32.6

[1] Credit is due Dr. C. R. Adair, for the milling of these samples.
[2] Obtained through the courtesy of the James and Harwell Company, manufacturers of converted rice, Houston, Texas.

were ground and suitable amounts were weighed for vitamin analyses. The technique for the determination of thiamin and riboflavin has been described in previously mentioned papers and nicotinic acid was determined according to the method of Melnick (1942).

The thiamin, riboflavin, and niacin contents of six samples each of unmilled rough rice, converted milled rice, and unconverted milled rice are presented in Table I. Samples 7, 18, and 23 were from rice of the same lot and the same variety (Early Prolific) and processed in the laboratory. Samples C and D were from rice of the Rexora variety and from different lots, Sample 100 was from rice of the Nira variety, and all were obtained from a plant in Houston, Texas—the first two from the pilot plant and the latter from the manufacturing plant. The method of processing in the laboratory differed slightly in details from the method used in the plant, the differences being related to duration of vacuum application and exposure to hydraulic

TABLE II

DISTRIBUTION OF THIAMIN, RIBOFLAVIN, AND NIACIN IN RICE HULLS AND RICE BRAN BEFORE AND AFTER CONVERSION

	Thiamin				Riboflavin				Niacin			
	Hulls		Bran		Hulls		Bran		Hulls		Bran	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
C[1]	3.41	1.81	21.0	6.80	.620	.470	2.79	1.90	48.5	29.8	258.0	230.0
D[1]	2.62	1.18	20.0	7.72	.660	.557	2.41	1.01	45.0	33.0	201.0	170.0

[1] Obtained through courtesy of the James and Harwell Company, manufacturers of converted rice, Houston, Texas.

pressure. In all cases, the processing took place without reuse of the steeping water. Results of further work in progress will disclose the conditions of processing (duration and extent of vacuum and hydraulic pressure) under which the maximum retention of the water-soluble nutrients occurs. The data show that this process has a favorable influence on the thiamin, riboflavin, and niacin content of the milled rice.

Whereas with the old method the retention of thiamin amounted to 22.2% (Sample 7), a retention of 63.5% was obtained after conversion; thus the difference was 41.3% in favor of the conversion. Such differences varied from 34.3% with Sample 18 to a difference of 63.5% with Sample D. The riboflavin showed less difference in retention before and after conversion. The range was from 15.8% in Sample 23 to 33.9% in Sample 7. The conversion process also brought

about a greater difference in retention of niacin, which ranged from 30% in Sample C to 61.2% in Sample 23. All these differences favored the conversion method.

In Table II, data are shown on the distribution of thiamin, riboflavin, and niacin in rice hulls and rice bran before and after conversion. The thiamin content of rice hulls of Lot C was 3.41 μg per gram before conversion and 1.81 μg per gram after conversion. The thiamin content of rice bran was 21.0 μg before and 6.80 μg per gram after processing, which indicates that the thiamin was lost from the hulls and bran layers. Similar observations can be made for riboflavin and niacin in this lot and for all these vitamins in Lot D. It is evident from these data that losses of these water-soluble vitamins occurred in the outer layers of the kernels.

Summary

The thiamin, riboflavin, and niacin content of rice was determined before and after conversion and it has been found that this method of processing favors the retention of these vitamins.

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BROMINE RESIDUES FROM METHYL BROMIDE FUMIGATION OF CEREAL PRODUCTS

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(Received for publication December 14, 1942)

The increasing use of methyl bromide as a fumigant for cereal products raises the question of a residue in the fumigated product. Stenger, Shrader, and Beshgetoor (1939), working with laboratory fumigation, report a gain of 91 parts per million in bromine [1] content

[1] The word "bromine" is used throughout this paper to refer to the element without regard to the form in which it was present in the samples that were analyzed.

of whole-wheat flour due to fumigation. Neither the dosage nor the time of exposure is given, but both were probably in the range of fumigation practice.

Dudley,[2] Miller, Neal, and Sayers (1940) report the bromine content of white flour as 111 ppm and of whole-wheat flour as 74 ppm following laboratory fumigation with a dosage of 2 pounds per 1,000 cubic feet and an exposure of 24 hours. They also report the results of commercial fumigations in which the bromine content of white flour ranged from about 47 to 72 ppm when the dosage was 1 pound per 1,000 cubic feet and the exposure 24 hours. In the latter case the samples were taken from the mill 48 hours after completion of the fumigation.

Roehm, Shrader, and Stenger (1942) report the total bromine content of commercially fumigated white flour as 23, 25, and 28 ppm with dosages of 0.083, 0.125, and 0.104 pound of methyl bromide per 1,000 pounds of load at temperatures from 51° to 84°F and an exposure of 74 hours.

Laug (1941) reports a bromine content of 411 ppm in flour 48 hours after a 24-hour laboratory fumigation with a dosage of 6.9 volume percent of methyl bromide (equivalent to 16.3 pounds per 1,000 cubic feet).

The purpose of the present investigation was to determine the quantity of residual bromine following the use of methyl bromide in various commercial fumigation practices. There are in general four ways in which methyl bromide is used as a fumigant for cereal products. First, mills and warehouses are fumigated as a whole (usually at a dosage of 1 to 2 pounds of methyl bromide per 1,000 cubic feet of space); second, vaults of varying size are used for fumigating infested products or containers such as returned bags; third, infested material is stacked and fumigated under tarpaulins; and fourth, cereal products are loaded into box cars and fumigated, the box cars in a sense serving as fumigation vaults. Under all four methods of actual commercial practice, samples of flour or other commodities were collected and analyzed for bromine content.

Analytical Procedure

Several methods for determining small amounts of bromine in biological materials, fruits and vegetables, flour, and other foodstuffs have been published in recent years (see Literature Cited). The procedure that we have used in applying the methods to cereal and forage products is as follows:

A 10-g sample, ground if necessary, is weighed out in a porcelain dish and treated with 3 ml of saturated sodium chloride solution and

[2] Dudley and Neal (1942) have more recently determined methyl bromide residues in various classes of fumigated products and discussed their relation to consumer hazard.

40 ml of 2% alcoholic solution of potassium hydroxide and mixed thoroughly. After evaporation of the alcohol and drying on a steam bath, the sample is ashed in a muffle furnace at 250°C. The door of the muffle is kept slightly open to permit escape of smoke, and when the smoking has almost ceased the temperature is raised to 400°C and finally, after 30 minutes, it is controlled at 500°C for 45 to 60 minutes. At no time should this final temperature be exceeded.

After being cooled, the residue is extracted with two 25-ml portions of 1 : 50 hydrochloric acid. The extracts are filtered through a folded filter, the residue is transferred to the filter with 10 ml of water, and both residue and paper are washed with two additional 10-ml portions of water, the filtrates being caught in a 500-ml Erlenmeyer flask. The filter paper is returned to the dish and treated with 3 ml of the sodium chloride solution and 10 ml of alcoholic potassium hydroxide. The drying and ignition are repeated as before, except that the ashing can be started at 400°C. The residue is extracted with 25 ml of the dilute hydrochloric acid, and the washings are repeated as before, the filtrates being caught in the same flask.

The combined filtrates are neutralized with 2% aqueous sodium hydroxide, using methyl orange as indicator, and evaporated to about 100 ml. To the solution are added about 1 g of sodium dihydrogen phosphate, 5 ml of sodium hypochlorite solution (1*N* NaOCl in 0.1*N* NaOH), and 5 ml of 2% sodium hydroxide. The mixture is

TABLE I

BROMINE CONTENT OF UNFUMIGATED CEREAL AND FORAGE PRODUCTS

Material	Bromine
	ppm
Oatmeal feed	21
Flour	14
Alfalfa hay	13
Alfalfa-leaf meal	22
Corn meal	24
Corn flour	23
Wheat-germ meal	44
Rice bran	13
Rice polish	17
Mung beans	37

boiled gently for 10 minutes; after 1 minute 5 ml of cp sodium formate solution (50 g in water to make 100 ml) is introduced, and the boiling is continued for 10 minutes. The solution is cooled and treated with 1 drop of 1% sodium molybdate solution, 0.5 g of potassium iodide, and 10 ml of 12*N* sulfuric acid. Titration is made immediately with standard sodium thiosulfate, starch indicator being added just before the end point.

Cp chemicals are used throughout the procedure, and a reagent blank is determined. One milliliter of 0.01*N* thiosulfate is equivalent to 0.1332 mg of bromine. It should be remembered that this method includes iodine as well as bromine.

The average recovery of known amounts of potassium bromide added to 12 samples of different materials was 97.2%, with a high of 100.5 and a low of 94.8.

Before the amount of bromine residues resulting from fumigation could be determined, it was necessary to determine the bromine content of unfumigated materials. The results of analyses of unfumigated samples representing several classes of cereal and forage products are given in Table I.

Mill and Warehouse Fumigation

The data for two mill fumigations are presented in Table II. It was impossible to get complete information for mill A. Mill B is a modern mill of brick and concrete construction with tightly fitting

TABLE II

BROMINE CONTENT OF WHITE FLOUR IN MILL AND WAREHOUSE FUMIGATION

Mill	Temperature	Dosage	Position from which samples were taken	Total bromine	Bromine due to fumigation
	°F	oz per 1,000 cu ft		ppm	ppm
A	72–75	16	Check (not fumigated)	21	—
			First floor of warehouse	35	14
			Second floor of warehouse	36	15
B	70–75	13.6	Check (not fumigated)	18	—
			Seventh floor of mill	32	14
			Sixth floor of mill	38	20
			Fifth floor of mill	34	16
			Fourth floor of mill	34	16
			Third floor of mill	36	18
			Second floor of mill	38	20
			First floor of warehouse	44	26

steel window sashes; its capacity is 823,000 cubic feet. In mill B, 700 pounds of methyl bromide were used, piped into the mill as follows: 225 pounds to the basement and first floor, 190 pounds to the second and third floors, 135 pounds to the fourth and fifth floors, and 150 pounds to the sixth and seventh floors.

The door from each floor into the stair well was closed but not sealed. The mill was fumigated between 4:30 and 6 pm, and it was opened the following morning at 11 o'clock. Twenty-four hours later samples were collected in glass jars, as indicated in Table II.

The bromine contents of these samples were low, but entomologists cooperating with the authors reported better than 99% kill of the insects.

Vault Fumigation

Table III gives the data for a series of fumigations of oatmeal in two vacuum vaults of different size. In each fumigation the vault was evacuated to a pressure of 2 inches of mercury and the gas was recirculated for 15 minutes. Under these conditions the quantity of bromine retained by the fumigated product reached much higher levels than in the mill fumigations of flour, the maximum concentration being 247 ppm.

TABLE III

VACUUM VAULT FUMIGATION OF OATMEAL WITH METHYL BROMIDE

Capacity of vault	Oatmeal in vault	Exposure	Dosage		Temperature	Total bromine	Bromine due to fumigation
cu ft	*tons*	*hours*	*lbs per 1,000 cu ft*	*oz per ton*	*°F*	*ppm*	*ppm*
Check (not fumigated)					—	21	—
357.5	5.0	3	3	3.43	69	70	49
	5.9	3	4	3.88	72	113	92
	6.0	3	5	4.77	70	174	153
	1.0	3	3	17.2	70	40	19
	1.0	3	2	11.4	72	76	55
	6.0	4	5	4.77	68	105	84
	6.0	15	3	2.86	70	155	134
1,800	31.0	15	5	4.64	70	268	247
			2	1.86	70	139	118

Data on fumigation in an atmospheric vault of 800 cubic feet capacity are given in Table IV. In the first four fumigations two one-quart pasteboard cartons filled with wheat-germ meal were put in the vault along with the load. At the end of the fumigation one carton was allowed to stand three weeks and was therefore subjected to considerable aeration before its contents were analyzed, while the contents of the other were transferred to a glass jar to avoid loss of bromine through aeration.

The data on the linseed and cottonseed meal are included to show the rate of loss of bromine from fumigated materials of this nature.

Fumigation under Tarpaulins

Table V presents the data for tarpaulin fumigation. The relatively high dosages used caused a large quantity of bromine to be left in the flour.

TABLE IV

ATMOSPHERIC VAULT FUMIGATION OF VARIOUS CEREAL PRODUCTS

Commodity analyzed	Load in vault	Dosage		Temperature	Exposure	Total bromine[1]	Bromine due to fumigation
	tons	*oz per 1,000 cu ft*	*oz per ton*	*°F*	*hours*	*ppm*	*ppm*
		Check (not fumigated)		—	—	44	—
Wheat-germ meal[2]	1	15	12	80	12	(a) 113	69
						(b) 106	62
	2	20	8	80	12	(a) 118	74
						(b) 113	69
	2	25	10	74	12	(a) 112	68
						(b) 122	78
	1	10	8	74	16	(a) 93	49
						(b) 78	34
Wheat flour	1	Check (not fumigated)		—	—	23	—
		20	16	78	12	45	22

Commodity analyzed	Dosage	Exposure	Time held before analysis	Total bromine
	oz per ton	*hours*		*ppm*
Cottonseed meal	3	3	1 hour	144
			24 hours	74
			7 days	67
Linseed meal	3	3	1 hour	108
			24 hours	68
			7 days	60

[1] Samples transferred to jar indicated by (a); samples held in carton after fumigation indicated by (b).

[2] The vault was loaded with corn flour in the first three fumigations and with corn meal in the fourth. Wheat-germ meal in a pasteboard carton was also put in vault.

TABLE V

FUMIGATION UNDER TARPAULINS

Commodity	Quantity	Exposure	Dosage	Total bromine	Bromine due to fumigation
	tons	*hours*	*oz per ton*	*ppm*	*ppm*
Wheat flour	7	20	4.5	92	77
				99	84
Mung beans	15	—	3.2	81	44
Check on wheat flour		—	—	15	—
Check on mung beans		—	—	37	—

Box Car Fumigation

A box car loaded with 100,000 pounds of wheat flour was fumigated with 10 pounds of methyl bromide and then shipped from Kansas City to Chicago. On its arrival samples of flour were sent to Washington for analysis. The car had been 3 days in transit and the temperature was approximately 90°F. The flour was found to contain 62 ppm of

bromine. A sample of the same flour taken 4 weeks later showed 60 ppm of bromine and a third sample after another week also analyzed 60 ppm. No check was taken.

Summary

Cereal products that had been fumigated with methyl bromide were analyzed to determine what bromine residues might be expected from such treatments. Samples were taken after commercial fumigations by four methods—(1) fumigation of the entire mill or warehouse, (2) fumigation in vacuum or atmospheric vaults, (3) fumigation under tarpaulins, and (4) fumigation of box cars. The results indicate that commercial fumigation practices can be expected to increase the bromine content of flour and other cereal products. The increases ranged from practically nothing to 247 ppm, or nearly 12 times the amount originally present. In most cases, however, the fumigated product contained less than twice the original amount. Vacuum vault fumigation apparently caused larger bromine residues than other practices.

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THE USE OF STATISTICAL METHODS IN QUALITY EVALUATION OF BARLEY AND MALT DATA[1]

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(Read at the Annual Meeting, May 1942)

In the quality evaluation of certain agricultural crops, limitations imposed by the physical capacity of the laboratory, or the nature of the samples available, often make it necessary to study a sample which is a composite of several replicated plots or a composite of a large number of samples from a given area. This is especially true when such data are obtained for a number of stations, or locations, over a period of years. The purpose of this paper is to illustrate the use and the interpretation of certain statistical methods in the analysis of data of this type. Wherever possible, however, it is desirable (for each factor studied) to make separate determinations for each replicate in place of one determination on the composite sample.

The "Barley and Malt Laboratory" at Madison since 1934 has evaluated, for different barley and malt characters, a number of standard barley varieties grown at representative experiment stations in the North-Central and Western states. Physical limitations of the laboratory necessitated the use of composite samples from the replicated plots. The data used in the present study are for 5 varieties grown at 6 stations during the 4-year period, 1935 to 1938. The original data have been presented by Dickson *et al* (1940, 1942). The stations included in the study reported here were East Lansing, Mich., Urbana, Ill., Madison, Wis., Waseca, Minn., Kanawha, Ia., and Brookings, S. D.

The interpretation of the large mass of data present problems in the statistical analysis. The data were studied first by the analysis of variance, using the three-factor interaction, variety × station × year, as the estimate of the experimental error (Dickson *et al* 1940). In order to obtain additional information on ranking of the varieties for the various factors at the different stations and in the different years, the

[1] Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station. The cooperative investigations include the agricultural experiment stations of California, Colorado, Illinois, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, South Dakota and Wisconsin, where the uniform barley varietal series were grown. The Federal WPA has contributed to the research program through a grant under the University of Wisconsin WPA Natural Science Project. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

[2] Assistant Professor in Agronomy, and Professor of Plant Pathology, University of Wisconsin and Agent, Division of Cereal Crops and Diseases. The authors wish to thank Dr. Churchill Eisenhart for his counsel in selecting the methods of statistical analysis, for deriving the *t* tests appropriate to testing individual interactions, and for his assistance in the preparation of the manuscript.

data were analyzed subsequently (1942) in more detail by two additional methods which differ somewhat from each other.

The two additional methods of analysis are illustrated here, using only a part of the data presented in the analysis of variance. Diastatic power of malt, yield of grain in bushels per acre, and protein content of barley are used to show the comparison between the three methods of analysis.

The variety × station and the variety × year interactions were separated into their component parts. The separation of the variety × station interaction into its 30 components provides a measure of the discrepancy of the actual reaction of each variety at each station from an expected value based on both the reactions of the variety and station in question. The variety × year interaction is analyzed in a similar manner.

The linear regression between the varietal mean at each station with the mean of all five varieties at each station was calculated for each variety. The regression analysis determines whether there is any difference among varieties in the reaction, for the character under consideration, in relation to the mean reaction of all varieties from stations with low values to those with high values. For example, as will be seen later, the spread in the diastatic power of Oderbrucker in relation to that of Wisconsin Barbless is much greater at stations with a high mean diastatic power than those with a low mean. This latter method, therefore, does not measure exactly the same thing as the former, although it offers a further check upon interpretation of the data.

The Use of the Analysis of Variance

The use of composite samples presented the problem of finding a valid estimate of experimental error. The three-factor interaction, variety × station × year, was used in all cases as the best available estimate of the experimental error. Before this was done, the mean squares for variety × year at each station and the mean squares for variety × station for each year were tested, respectively, for homogeneity by the use of Bartlett's test (1937). The tests for homogeneity showed that the variety × year interaction mean squares for the stations included in the present study and variety × station interaction mean squares for each year were relatively homogeneous. An analysis including the results from Bozeman, showed that the interaction for some of the characters were not homogeneous. The principles and tests applicable to this problem were discussed by Ozanne.[3] The F values for the several factors are given in Table I. All F values given

[3] P. R. Ozanne: Unpublished paper prepared in conjunction with Dr. C. Eisenhart, University of Wisconsin, 1940.

in this table were obtained by using the three-factor interaction, variety × station × year, as the experimental error. The variety mean squares were compared with the three-factor interaction variety × station × year, mean squares in place of variety × station mean squares for two reasons. In the first place, as pointed out by Yates

TABLE I

ANALYSIS OF VARIANCE FOR FIVE BARLEY VARIETIES (ODERBRUCKER, MANCHURIA, WISCONSIN BARBLESS, VELVET AND TREBI) GROWN AT SIX STATIONS (EAST LANSING, MICH., URBANA, ILL., MADISON, WIS., WASECA, MINN., KANAWHA,[1] IA., AND BROOKINGS, S. D.) FOR FOUR YEARS (1935 TO 1938)

Factors for which F values were determined	Source of variation with F values						Error mean square
	Varieties	Years	Stations	Varieties × stations	Varieties × years	Stations × years	
Yield of barley per acre,[2] *bu*	68.5	40.8	178.4	2.9	3.6	40.9	12.74
Test weight of barley, dry basis, *lb*	2.7	30.0	100.4	2.1	1.8	35.6	1.47
Kernel weight of barley, dry basis, *mg*	97.9	16.8	65.7	0.8	0.7	13.1	2.81
Hull content of barley, dry basis, %	5.1	74.7	15.3	0.7	1.2	6.4	1.90
Ash content of barley, dry basis, %	16.3	83.7	32.4	0.7	1.7	9.4	0.017
Protein content of barley, dry basis, %	10.4	5.7	55.6	0.7	0.8	9.1	0.75
Time required in steep to reach 46% moisture, est., *hr*	25.2	6.2	45.8	1.7	3.6	16.2	23.96
Recovery of malt from barley, dry basis, %	9.9	39.2	8.2	0.6	1.9	4.1	2.45
Extract content of malt, fine grind, dry basis, %	18.2	39.0	108.5	0.6	1.4	21.5	1.065
Conversion time, *min*	13.0	1.8	5.0	1.6	0.9	4.1	5.83
Diastatic power of malt, °*L*	56.0	7.9	37.0	1.6	2.2	9.6	339.50
Ratio, wort nitrogen to malt nitrogen, %	75.3	14.5	5.9	0.8	0.9	5.4	4.33
Degrees of freedom	4	3	5	20	12	15	60[3]
0.05 level	2.52	2.76	2.37	1.75	1.92	1.83	—
0.01 level	3.65	4.13	3.34	2.26	2.50	2.36	—

[1] Barleys were grown at Emmetsburg, Ia., in 1935.
[2] Yields were not obtained at East Lansing, Mich., in 1938, values were interpolated.
[3] Variety × station × year interaction was used as error; see text.

and Cochran (1938), there is no reason to suppose that the varietal differences from station to station are the same for each pair of varieties. Secondly, in order to justify the comparison of the variety mean square with the variety × station mean square, each year a new set of stations selected at random from the entire area sampled should be used.

Separation of Varietal Interactions into Component Parts

A significant F for variety × station interaction indicates that certain of the varieties at some of the stations respond differently from certain other varieties. In order to determine which varieties reacted significantly different from expectation at the respective stations, the sums of squares for the variety × station interactions was separated into individual components measuring the discrepancy of the four-year mean of each variety at each station from that expected on the hypothesis of no interaction. The procedure followed was a modifica-

tion of the method described by Cochran (1937) and Yates and Cochran (1938).

The 20 degrees of freedom for the variety × station interaction were separated into 30 somewhat interdependent component parts, one for each variety-station comparison. Similarly, the 12 degrees of freedom for the variety × year interaction were separated into 20 somewhat interdependent component parts, one for each variety-year comparison. The analysis employed follows.

The deviation of the four-year mean response of the ith variety at the jth station $(V \times S)ij$ from that expected on the hypotheses of no interaction was obtained by the following equation:

$$(V \times S)ij = \bar{X}ij - \bar{X}i. - \bar{X}.j + \bar{X}.. \tag{1}$$

where $\bar{X}ij$ is the 4-year mean response of the ith variety at the jth station, $\bar{X}i.$ is the 4-year mean response of the ith variety at all 6 stations, $\bar{X}.j$ is the 4-year mean response of all 5 varieties at the jth station, $\bar{X}..$ is the 4-year mean response of all 5 varieties at the 6 stations. For example, the deviation of the diastatic power of Trebi malt at Kanawha from that expected on the basis of no interaction, using the data in Table III, is

$$146 - 156 - 148 + 168 = 10$$

The deviation of the actual values from the expected were tested for significance by the t test as below

$$t = \frac{(V \times S)ij}{\sqrt{\frac{\text{Error mean square}}{4} \cdot \frac{2}{3}}} \tag{2}$$

The degrees of freedom, 60, are those associated with the error mean square. The error mean square was divided by 4 because each variety value is the mean of 4 years' data. The factor 2/3 is a correction for the interdependence of the 4 quantities of which the numerator of equation 2 is a linear function. It will be noticed that the correction 2/3 is directly proportional to 20/30, namely, the degrees of freedom for variety × station (20) and the total number of variety × station comparisons (30). The formal explanation is as follows: when $i = 1$ and $j = 1$, equation 1 can be rewritten in the following manner:

$$(V \times S)_{11} = \frac{\bar{X}_{11} - \bar{X}_{11} + \sum_{j=2}^{6} \bar{X}1j}{6} - \frac{\bar{X}_{11} + \sum_{i=2}^{5} \bar{X}i1}{5} + \frac{\bar{X}_{11} + \sum_{i=2}^{5} \bar{X}i1 + \sum_{j=2}^{6} \bar{X}1j + \sum_{i=2}^{5} \sum_{j=2}^{6} \bar{X}ij}{30} \tag{3}$$

where $(V \times S)_{11}$ is the deviation of the actual value of the 4-year mean of variety 1 at station 1, $\bar{X}_{11}$, from the expected value based on no interaction, $\sum_{j=2}^{6} \bar{X}1j$ is the sum of the 4-year mean response of variety 1 at the other 5 stations, $\sum_{i=2}^{5} \bar{X}i1$ is the sum of the 4-year mean responses of the other 4 varieties at station 1, and $\sum_{i=2}^{5} \sum_{j=2}^{6} \bar{X}ij$ is the sum of the 4-year mean responses of varieties 2 to 5 at stations 2–6. The right side of equation 3 reduces to

$$\bar{X}_{11}\left(1 - \frac{1}{6} - \frac{1}{5} + \frac{1}{30}\right) - \sum_{j=2}^{6} \bar{X}1j\left(\frac{1}{6} - \frac{1}{30}\right) - \sum_{i=2}^{5} \bar{X}i1\left(\frac{1}{5} - \frac{1}{30}\right) + \sum_{i=2}^{5} \sum_{j=2}^{6} \bar{X}ij\left(\frac{1}{30}\right) \quad (4)$$

Simplifying equation 4 we get,

$$\bar{X}_{11}\left(\frac{20}{30}\right) - \sum_{j=2}^{6} \bar{X}1j\left(\frac{4}{30}\right) - \sum_{i=2}^{5} \bar{X}i1\left(\frac{5}{30}\right) + \sum_{i=2}^{5} \sum_{j=2}^{6} \bar{X}ij\left(\frac{1}{30}\right) \quad (5)$$

The variance of $(V \times S)_{11}$ in relation to error variance is then,

$$\text{variance } (V \times S)_{11} = \left[\left(\frac{20}{30}\right)^2 + \left(\frac{5}{30}\right)^2 4 + \left(\frac{4}{30}\right)^2 5 + \left(\frac{1}{30}\right)^2 4 \cdot 5\right] [\text{error variance}] \quad (6)$$

which reduces to

$$\text{Variance } (V \times S)_{11} = \frac{1}{(30)^2}[400 + 80 + 100 + 20](\text{error variance}) = \frac{2}{3}(\text{error variance})$$

Since similar steps can be carried out for any pair of values i and j, the result is true generally. In order to determine how large a difference was necessary between the actual and expected values to be significant at the 0.05 (or 0.01) level of significance, equation 2 was written as below,

$$(V \times S)ij = [t.05 \text{ (or } t.01) \text{ for 60 d. f.}] \cdot \sqrt{\frac{\text{Error mean square}}{4} \cdot \frac{2}{3}} \quad (8)$$

For example, the difference necessary for significance at the 0.01 level between the observed and expected diastatic power of malt is

$$2.66 \sqrt{\frac{339.50}{4} \cdot \frac{2}{3}} = 20$$

The interaction variety × year was analyzed in a similar manner; the deviation of the mean response at all stations for each year from that expected on the basis of no interaction was determined by equation 9:

$$(V \times Y)ik = \bar{X}ik - \bar{X}i. - \bar{X}.k + \bar{X}.. \qquad (9)$$

where $\bar{X}ik$ is the mean response at all 6 stations of the ith variety in the kth year, $\bar{X}i.$ is the mean response at all stations of the ith variety for all 4 years, $\bar{X}.k$ is the mean response at all 6 stations of all 5 varieties in the kth year, and $\bar{X}..$ is the mean response of 5 varieties at the 6 stations for the 4 years. The $(V \times Y)ik$ values were tested for significance as below

$$t = \frac{(V \times Y)ik}{\sqrt{\frac{\text{Error mean square}}{6} \cdot \frac{3}{5}}} \qquad (10)$$

It will be noticed that the correction 3/5 is directly proportional to 12/20, namely the degrees of freedom for variety × year (12) and the total number of variety × year comparisons (20).

TABLE II

INTERACTION OF THE FIVE BARLEY VARIETIES AT THE SIX STATIONS (DATA FOR THE FOUR YEARS COMBINED) AND IN THE FOUR YEARS (DATA FOR THE SIX STATIONS COMBINED).

(The F values for interactions compared with the number of significant interactions determined at the 0.05 and 0.01 levels. See Table I for stations and years included.)

Factors	Interaction based on analysis of variance, Table I			Number significant varietal interactions[1]				Coefficient of variability
	Error mean square	F value varieties × stations	F value varieties × years	Varieties × stations		Varieties × years		
				0.05	0.01	0.05	0.01	
Yield of barley, per acre, *bu*	12.74	2.9	3.6	9	3	5	4	10.6
Test weight barley, dry basis, *lb*	1.47	2.1	1.8	7	2	3	1	3.2
Kernel weight barley, dry basis, *mg*	2.81	0.8	0.7	1	0	1	0	6.5
Protein content barley, %	0.75	0.7	0.8	0	0	0	0	5.2
Time required in steep to reach 46% moisture, *hr*	23.96	1.7	3.6	4	2	6	3	15.7
Recovery of malt from barley, dry basis, %	2.45	0.6	1.9	0	0	3	1	1.9
Extract content malt, dry basis, %	1.06	0.6	1.4	0	0	3	1	1.4
Diastatic power malt, °*L*	339.50	1.6	2.2	4	1	3	1	11.0
Ratio wort nitrogen to malt nitrogen, %	4.33	0.8	0.9	1	0	0	0	6.1
F value 0.05 level		1.75	1.92					
F value 0.01 level		2.20	2.50					

[1] Determined by modification of the Yates and Cochran method described in the text.

The number of components for both variety × station and variety × year comparisons which exceed the 0.05 and 0.01 levels of significance are tabulated in Table II.

An examination of the data in Table II shows that there is a fair agreement of the F value for the interactions variety × station and variety × year with the number of varieties which show a significant departure from the values expected on the basis of no interaction. For

TABLE III

FOUR-YEAR MEAN DIASTATIC POWER OF MALT, MEAN YIELD IN BUSHELS PER ACRE AND MEAN PROTEIN CONTENT OF BARLEY OF EACH OF THE FIVE BARLEY VARIETIES AT EACH OF THE SIX STATIONS

(Interactions exceeding 0.05 single starred, exceeding 0.01 double starred; + or − indicates value greater or less than expected on basis of no interaction)

Variety	Station						Variety mean
	East Lansing	Urbana	Madison	Waseca	Kanawha	Brookings	
DIASTATIC POWER OF MALT							
Trebi	146	135	129	157	154	213	156
Wisconsin Barbless	128*+	130	110	133	134	155**−	131
Velvet	136	156	143	163	162	201	160
Oderbrucker	151*−	174	182	175	211	254*+	191
Manchuria	178	187	185	195	208	253	201
Station mean	148	156	150	165	174	215	168
YIELD OF BARLEY, BUSHELS PER ACRE							
Trebi	31.3**−	51.5*+	30.1*−	60.1*+	37.5	28.2	39.8
Wisconsin Barbless	38.0	43.2**−	33.9	57.8	36.4	25.4	39.1
Velvet	34.0*+	40.0	26.3	47.1*−	34.0	21.0	33.7
Oderbrucker	25.9	40.0*+	23.6	39.7**−	22.4	15.0	27.8
Manchuria	22.2	36.0	20.5	45.2	24.1	14.4	27.1
Station mean	30.3	42.1	26.9	50.0	30.9	20.8	33.5
PROTEIN CONTENT OF BARLEY							
Treib	12.4	12.2	12.3	12.9	13.9	15.8	13.2
Wisconsin Barbless	13.0	12.7	12.9	13.0	13.9	16.5	13.7
Velvet	12.9	13.1	13.5	13.8	14.3	16.3	14.0
Oderbrucker	12.6	13.6	14.1	14.0	14.8	18.0	14.5
Manchuria	13.4	13.5	14.0	14.9	14.7	17.1	14.6
Station mean	12.9	13.0	13.4	13.7	14.3	16.7	14.0

the interactions variety × station and variety × year there are respectively 30 and 20 comparisons. For yield of barley in bushels per acre the F value for both interactions exceeds the 0.01 level of significance. There are 9 and 5 individual interactions exceeding the

0.05 level, of which 3 and 4 exceed the 0.01 level, respectively, for variety × station and variety × year interactions. Interactions which approach the 0.05 level, such as steeping time and diastatic power for variety × station, have 4 individual comparisons each exceeding the 0.05 level and 2 and 1, respectively, exceeding the 0.01 level. The interactions, which have an F value near 1, show at the most only one individual varietal interaction exceeding the 0.05 level.

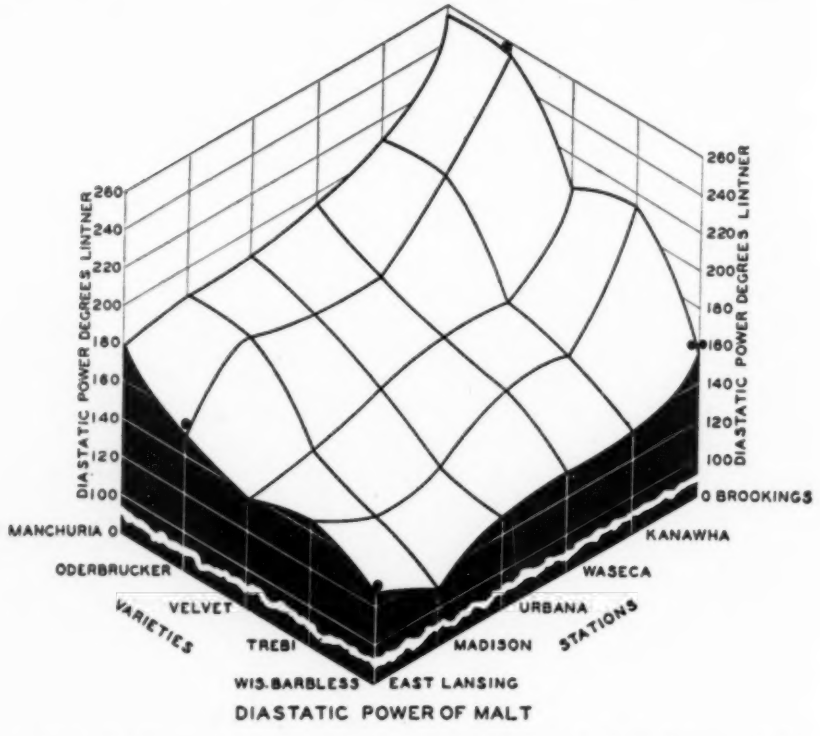

Fig. 1. Graphic comparison of diastatic power of the malts from the five varieties grown at the five stations in 1935 to 1938. One and two black dots indicate, respectively, interactions exceeding the 0.05 and 0.01 levels of significance.

In the interpretation of the data presented above, considerable care must be exercised. As shown in Table II there are in most cases individual comparisons which exceed the 0.05 and in a few instances several which exceed the 0.01 level of significance where the F value for the interaction of the combined comparisons is less than the 0.05 level. It is reasonable to expect, with the number of individual comparisons made (30 and 20) that certain of these would exceed the 0.05 level due to chance alone when no difference exists. Also since the 0.05 level is after all only an arbitrary division between significance and non-

significance, the difference in significance of an interaction slightly less than, and one slightly greater than, the 0.05 level is small. For these reasons in the interpretation of the data given in Table II, the individual interactions which exceed the 0.01 level are considered as sig-

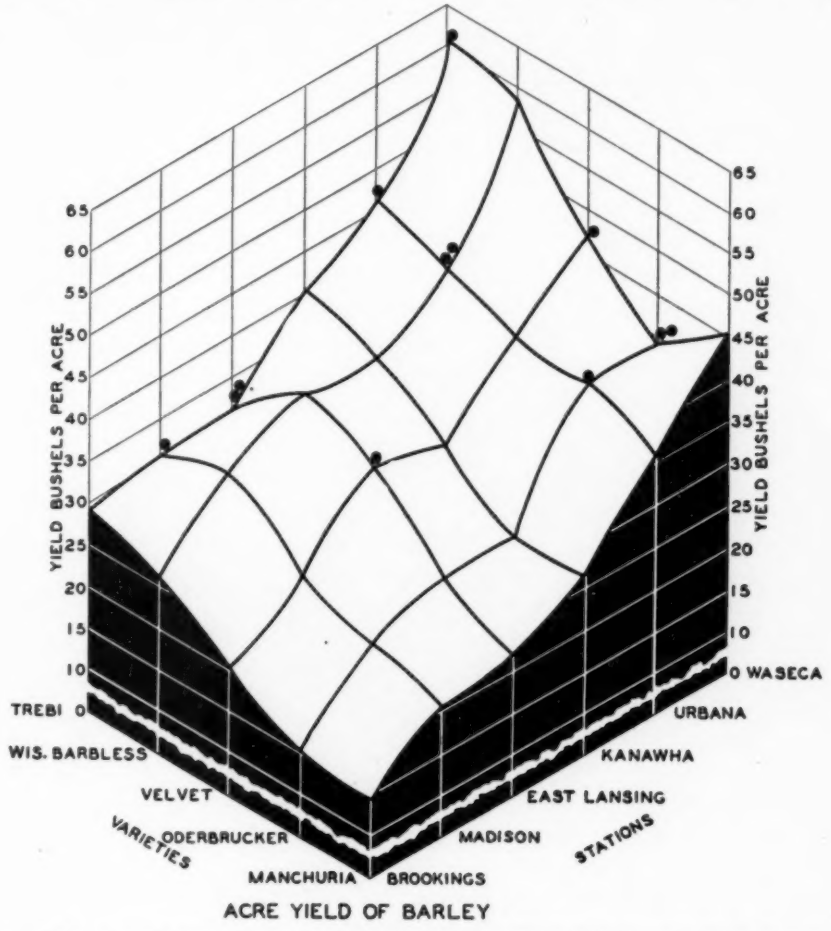

Fig. 2. Graphic comparison of acre yield of barley for the five varieties grown at the six stations in 1935 to 1938. One and two black dots indicate, respectively, interactions exceeding the 0.05 and 0.01 levels of significance.

nificant, while those between the 0.05 and 0.01 level are considered as being indicative of possible significance.

The four-year means of each variety at each station for diastatic power of malt and bushel yield and protein content of barley are given in Table III. The data are shown in graphic form in Figures 1 to 3. The diastatic power of Wisconsin Barbless at Brookings was 155, which is significantly less than the expected value of 178 based on no inter-

action. Other variety × station comparisons which are indicative of significance were Wisconsin Barbless at East Lansing (128, 111) Oderbrucker at East Lansing (151, 171), and Oderbrucker at Brookings (254, 238). The first number in the brackets refers to the actual value, the second to that expected on the basis of no interaction.

For yield, three comparisons, Trebi at East Lansing (31.3, 36.6), Wisconsin Barbless at Urbana (43.2, 47.7) and Oderbrucker at Waseca (39.7, 44.3) exceeded the 0.01 level. Six other comparisons were in-

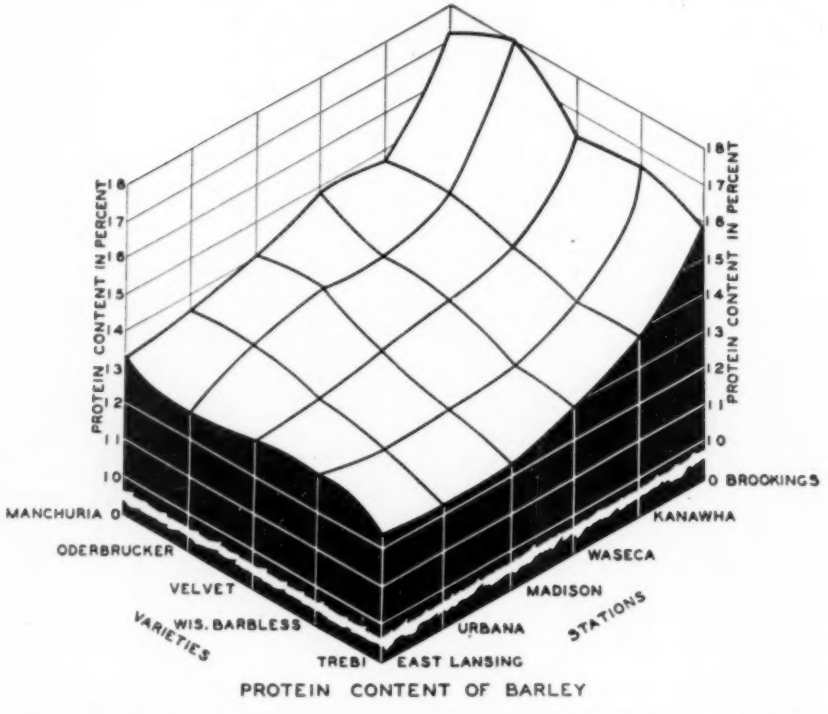

Fig. 3. Graphic comparison of protein in barley for the five varieties grown at the six stations in 1935 to 1938. Note that there are no significant interactions.

dicative of possible significance. In regard to protein content, none of the individual comparisons deviated significantly from that expected.

The four-year means of each variety at each station are presented graphically in Figures 1 to 3. In constructing the figures the varieties were arranged along one axis and stations along the other. The functions of the varietal reactions for a given factor at a given station form a series of lines perpendicular to a base plane common to both varieties and stations. The relations between varieties and stations for the given factor are then observed over the entire range of the stations

included. The arrangement of the varieties and stations along the axis was in ascending order for the factor means, purely as a method of keeping the tops of the perpendicular lines in view in the three-dimensional graphs. Likewise, purely for clarity of presentation, the tops of the vertical lines representing varietal reaction were connected for the individual varieties at the different stations and for the five varieties at each station. In so doing the interaction of varieties × stations is represented as a sectional surface or plateau. The extent of interaction is then expressed by the elevations and depressions in the surface of the plateau. The points on the plateau where the interactions are significant at the 0.05 and 0.01 levels of significance, as determined from the data in Table III, are indicated by one and two black dots respectively. A regular surface of the plateau indicates that the varieties maintained the same relative ranking at the different stations. The influence of variety and station upon the reaction of the factor is shown by the rise in the plateau along the two axes, *i.e.*, varieties and stations.

Regression Analysis

The relationship between varietal differences and the environmental conditions, at the different stations, affecting diastatic power of malt and grain yield and protein content of barley was investigated further by regression analysis using the method described by Yates and Cochran (1938). In accordance with this method, for each of the 5 varieties the linear regression was calculated between the 4-year mean responses of that variety at the several stations and the 4-year mean responses of all varieties at the same stations. As stated by Yates and Cochran, the object of taking the regression on the mean response of all 5 varieties rather than the response of the remaining 4 varieties is to eliminate a spurious component of regression which will otherwise be introduced into the experimental error.

In brief, the procedure followed consisted of (1) separating out, from the 20 degrees of freedom for variety × station interaction, the 4 degrees of freedom measuring the differences among the several regressions and (2) testing these for significance.

The first step is to obtain the sums of squares for the station means about the general mean from the data given in Table III, by the formula

$$\sum_{1}^{6} (\bar{X}.j - \bar{X}..)^2 \qquad (11)$$

where $\bar{X}.j$ is the 4-year mean of all 5 varieties at the jth station and $\bar{X}..$ is the general mean. The values $\bar{X}.j - \bar{X}..$ for all 3 characters studied are given in Table IV.

TABLE IV

DEVIATIONS OF STATION MEAN YIELDS FROM THE GENERAL MEAN (UPPER SECTION) AND VARIETAL TOTALS AT EACH STATION MULTIPLIED BY THE DEVIATIONS OF THE STATION MEAN YIELDS FROM THE GENERAL MEAN (BELOW) FOR DIASTATIC POWER OF MALT AND GRAIN YIELD AND PROTEIN CONTENT OF BARLEY

	$(X.j - X..)$		
Station	Diastatic power of malt	Grain yield of barley	Protein content of barley
East Lansing, Mich.	−20	−3.2	−1.1
Urbana, Ill.	−12	+8.6	−1.0
Madison, Wis.	−18	−6.6	−0.6
Waseca, Minn.	−3	+16.5	−0.3
Kanawha, Iowa	+6	−2.6	+0.3
Brookings, S. Dak.	+47	−12.7	+2.7
$\sum_{j=1}^{6}(\bar{X}.j - \bar{X}..)^2$	3122	568.06	10.04
Variety		$\sum_{j=1}^{6}(\bar{X}.j - \bar{X}..)Tij$	
Trebi	14340	2718.83	38.77
Wisconsin Barbless	6298	2253.40	40.16
Velvet	11165	1932.65	35.23
Oderbrucker	17048	2048.86	51.84
Manchuria	13778	2415.23	38.00

The sums of squares for diastatic power of malt for Trebi using equation 11 are

$$[(-20)^2 + (-12)^2 + (-18)^2 + (-3)^2 + (+6)^2 + (+47)^2] = 3122$$

For each variety the totals for the 4-year period at each station are then multiplied by their respective $(\bar{X}.j - \bar{X}..)$ deviations and summed

$$\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij \qquad (12)$$

where Tij is the 4-year total of the ith variety at the jth station. The values thus obtained are the sums of products of the station means for all varieties and the station totals for a particular variety. These values are listed in the lower half of Table IV. As an illustration the value for diastatic power of malt for Trebi was obtained for 12 as follows:

$$\sum [(-20)(583) + (-12)(538) + (-18)(517) + (-3)(628) + (6)(616) + (47)(850)] = 14340$$

This can be obtained approximately from the data in Table III by using the variety × station means multiplied by 4; the result will not agree exactly with those above because of the rounding in determining the means in Table III.

The regression coefficients for the different varieties listed in Table V were obtained as follows,

TABLE V

REGRESSION COEFFICIENTS BETWEEN THE 4-YEAR MEAN RESPONSE OF A VARIETY AT EACH STATION AND THE MEAN RESPONSE OF ALL VARIETIES AT EACH STATION, FOR EACH VARIETY FOR DIASTATIC POWER OF MALT AND GRAIN YIELD AND PROTEIN CONTENT OF BARLEY

Variety	Diastatic power of malt	Grain yield of barley	Protein content of barley
Trebi	1.1483	1.1966	0.9654
Wisconsin Barbless	0.5043	0.9917	1.0000
Velvet	0.8941	0.8505	0.8772
Oderbrucker	1.3652	0.9017	1.2908
Manchuria	1.1033	1.0629	0.9462
Difference required 0.05	0.4663	0.2117	0.3866
for significance 0.01	0.6204	0.2817	0.5142

$$bi = \frac{\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij}{4\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2} \tag{13}$$

which for diastatic power of Trebi is $\frac{14340}{4(3122)} = 1.1483$

The sums of squares for the 4 degrees of freedom measuring the differences in the regressions of the 5 varieties is obtained from

$$\left[4\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2\right]\sum_{i=1}^{5} (bi - \bar{b})^2 \tag{14}$$

where the multiplier is needed to make this sum of squares comparable to those involved in Tables I and II, since the variance of a single b is $\frac{\sigma^2}{4}\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2$, where σ^2 is the error variance of a single observation, the factor 4 arising from the fact that b is a regression of means of 4. The following form of equation 14 is convenient for calculation,

$$\frac{\sum_{i=1}^{5}\left[\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij\right]^2 - \left\{\frac{\sum_{i=1}^{5}\left[\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij\right]}{5}\right\}^2}{4\sum_{j=1}^{6}(\bar{X}.j - \bar{X}..)^2} \tag{15}$$

which for diastatic power of the malt is:

$$\frac{\sum_{1}^{5} (14340)^2 + \cdots\cdots\cdots\cdots + (13778)^2 - \dfrac{(62629)^2}{5}}{(3122)\ (4)} = 5280.82$$

In Table VI are given the results of the above analyses. The sums of squares for the variety × station interactions based on 20 degrees of freedom are separated into 2 parts, the differences among the regressions and the deviations about the individual regressions, based on 4 and 16 degrees of freedom respectively.

TABLE VI

ANALYSIS OF VARIANCE FOR DIFFERENCES AMONG VARIETAL REGRESSIONS FOR DIASTATIC POWER OF MALT AND GRAIN YIELD AND PROTEIN CONTENT OF BARLEY

Source of variation	Degrees of freedom	Diastatic power of malt		Grain yield of barley		Protein content of barley		F	
		Mean square	F	Mean square	F	Mean square	F	0.05	0.01
Differences of regressions	4	1320.21	3.89	42.41	3.33	1.04	1.39	2.52	3.65
Deviations from regressions	16	356.63	—	35.80	—	0.43	—	—	—
Error	60	339.50	—	12.74	—	0.75	—	—	—

In order to test the significance of the difference between any two regressions b_1 and b_2, the t value was obtained as below.

$$t = \frac{(b_1 - b_2)}{\sqrt{\dfrac{2S^2}{4\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2}}} \qquad (16)$$

where S^2 is error mean square. Equation 16 can be changed to the following in order to determine how large a difference is necessary between any two regressions to be significant at either the 0.05 or 0.01 level of significance.

$$b_1 - b_2 = [t.05 \text{ (or } t.01) \text{ for 60 d.f.}] \cdot \sqrt{\frac{S^2}{2\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2}} \qquad (17)$$

which for diastatic power of barley at the 0.01 level is

$$2.66\sqrt{\frac{339.50}{2\ (3122)}} = 0.6204$$

The regressions are shown in Figure 4.

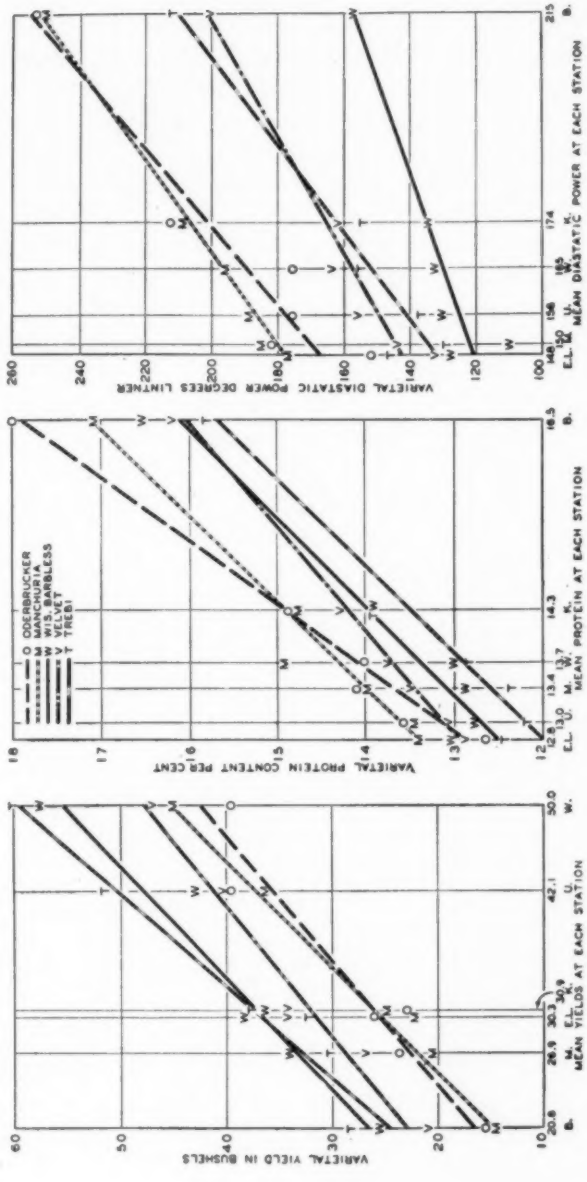

Fig. 4. Regressions for varieties against the means for all varieties for yield and protein content of barley and diastatic power of malt. The five varieties, Oderbrucker, Manchuria, Wisconsin Barbless, Velvet, and Trebi grown at East Lansing, Mich., Urbana, Ill., Madison, Wis., Waseca, Minn., Kanawha, Ia., and Brookings, S. D. Average of four years 1935 to 1938.

Regressions on mean yield of barley show the same response for Trebi as reported by Yates and Cochran (1938) for certain Minnesota data. Trebi has a greater tendency to increase in yield at the stations with the higher mean yields than the other varieties. This tendency, however, is only significant when compared with Velvet. The wide differences in the yielding capacity of the four varieties are shown by the relative position of the regression lines. No significant differences were found in the slope of the regression lines for protein content of barley.

The regression lines for diastatic power of malt show a marked difference between Wisconsin Barbless as compared to the other varieties. The increase in diastatic power of Wisconsin Barbless at the stations with the higher diastatic power is significantly less than that found for the other varieties with the exception of Velvet. Between Velvet and Oderbrucker the difference is significant.

The generally low interaction between varieties × station and varieties × years is especially important in relation to quality evaluation and in applying this information to a breeding program. The analyses indicate that for most factors studied the varieties maintain relatively the same ranking at the different stations and in the different years. This suggests that the relative ranking of the varieties and selections for most of the important quality factors can be determined reliably at a few representative stations.

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REPORT OF THE 1942–43 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

W. H. HANSON, Chairman

Henkel Flour Mills, Detroit, Mich.

(Read at the Annual Meeting, May 1943)

The results reported by the committee for the past two years showed significant differences in the spread potentialities of cooky flours milled from white wheat varieties (Loving, 1942; Hanson, 1943). Factors which seem to be associated to a marked degree with spread behavior are viscosity and granulation. It is quite apparent that a coarsely milled flour will show greater spread characteristics, and in some instances may reflect a slight lowering of the viscosity.

Opinions were offered by the members of the committee that a cooky flour from the same geographical area might vary considerably in spread potentialities from one crop to another. It was therefore suggested that the collaborators recheck their results as reported for 1941–42 (Hanson, 1943) with flours from the same mills and white wheat varieties, with the object of grouping the flours according to the spread factor W/T as obtained by cooky measurements, and also of determining whether there is any association between granulation or viscosity and spread behavior.

TABLE I

ANALYSES OF FLOURS USED IN COOKY TESTS

Flour[1]	Ash (15% m.b.)	Protein (15% m.b.)	Viscosity		Granulation (Through 250-mesh, 60 minutes)
			No-time	1 hour digestion	
	%	%	*MacM*	*MacM*	%
A	0.385	7.65	35	57	84
B	0.400	7.42	24	38	78
C	0.395	7.05	21	33	89
D	0.420	7.47	30	46	70

[1] Flour A was from New York State, flours B and C from Michigan, and flour D from the Pacific Coast.

Instructions to the mills supplying these flours were in accordance with the usual specifications—0.41 to 0.42% ash, 7.5 to 8.0% protein, and a maximum viscosity of 35° MacMichael, as determined by the no-time method for unbleached flours of 100% extraction. The four flours selected are referred to as A, B, C, and D, the analyses of which are shown in Table I.

Cookies were baked by the laboratory cooky test and procedure formula described by Hanson (1943) and by the semicommercial baking method outlined in Table II. In the latter method, three formulas which vary in the quantities of sugar and shortening called for are used.

TABLE II

FORMULAS USED IN TESTING COOKY FLOURS BY SEMICOMMERCIAL PROCEDURE [1]

Mixing stage	Ingredients	Formulas		
		1	2	3
		g	g	g
I	Fine granulated sugar	1510	1250	1510
	Salt	23	23	23
	Shortening (B and C type)	752	752	376
II	Milk powder	114	114	114
III	Whole eggs	270	270	270
	Invert sugar	227	185	227
	Vanilla—concentrate	8	8	8
IV	Water	454	454	454
	Ammonium carbonate	8	8	8
V	Flour	2250	2250	2250
	Soda	17	17	17
	Anhydrous monocalcium phosphate	5	5	5
	Totals	5638	5336	5262

METHOD:
Stage I Cream 2 minutes on second speed
Stage II Cream 1 minute on second speed
Stage III Mix 1 minute on second speed
Stage IV Mix 1 minute on low speed
Stage V Mix 2 minutes on second speed
EQUIPMENT:
20-quart Hobart Mixer, No. A200
Wire-cut cooky divider
1¼-inch round dies
SCALING WEIGHT:
4 ounces per dozen

[1] Submitted by H. W. Putnam.

Discussion

The rank of the cooky flours from the calculated "spread factor" as recorded in Table III shows very good agreement on flour B. Flour A was ranked 4 by a large majority of the collaborators, whereas flour from the same mill and wheat type ranked 1 in the committee report of 1941–42. Flour C, which was definitely poor in the instance of the 1941–42 crop, showed some improvement in rank and compared favorably with flour D for the current crop. When a comparison is made between the "spread factors" obtained for two different crops, the study definitely indicates that variations exist in spread potential-

ities of flours milled from known white wheat varieties. The *W/T* factors obtained on cookies produced by the semicommercial baking formulas are shown in Table IV. The semicommercial baking method

TABLE III

RANK OF COOKY FLOURS FROM CALCULATED *W/T* FACTORS

(Rank 1 signifies the best spread, 4 the poorest.)

Sample	Collaborator					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
A	4	2	4	4	4	4
B	1	1	1	1	1	1
C	2	4	2	2	3	3
D	3	3	3	3	2	2

TABLE IV

W/T FACTORS OBTAINED ON COOKIES BAKED BY THE SEMICOMMERCIAL FORMULAS

Flours	Formulas (See Table III)		
	1	2	3
A	7.8	5.7	6.3
B	6.5	5.4	6.5
C	7.3	5.2	6.7
D	8.0	5.5	6.7

was very informative and, as illustrated in Figure 1, showed differences in spread behavior resulting from substantial reductions in sugar and shortening. Owing to the present trend in the restriction of ingredi-

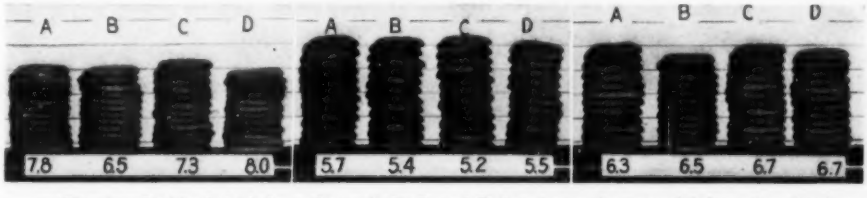

Fig. 1. Cookies baked from flours A, B, C, and D by the semicommercial formulas showing differences in the spread behavior resulting from variations in sugar and shortening. Groups at the left, center, and right, respectively, represent cookies baked by formulas 1, 2, and 3 described in Table II.

ents, the semicommercial test can be recommended for evaluating differences in cooky flours.

Summary

The laboratory and semicommercial baking methods employed showed considerable variations in the spread potentialities of the four

flours studied. The study has revealed that cooky flours may vary considerably from year to year in spread characteristics, although the analyses may be maintained fairly uniform during this time. The low viscosity white wheat flours respond very well to reductions in sweetening materials and shortener, provided that grinding and bolting operations in milling will increase the spread properties. Variations in spread are likely to occur when the total batter weight differs to the extent that it affects the bake-out loss owing probably to oven conditions.

Acknowledgments

The chairman wishes to express his thanks to the Victor Flour Mills of Pittsford, New York, and to the Centennial Flouring Mills of Tacoma, Washington, for the flours submitted from those states. To the members of this committee (C. E. Bode, T. E. Hollingshead, F. R. Schwain, H. M. Simmons, O. P. Skaer, H. W. Putnam, C. S. McWilliams, and E. P. Nelson) the chairman also wishes to express his appreciation and thanks.

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REPORT OF THE 1941-42 SUBCOMMITTEE ON TESTING CAKE FLOURS WITH BAKING-POWDER ADJUSTMENTS FOR ALTITUDE

JOSEPH DE HAAN, *Chairman*

General Mills, Inc., Spokane, Washington

(Read at the Annual Meeting, May 1942)

Barmore conducted a number of experiments at Fort Collins, Colorado, wherein the baking powder in the A.A.C.C. cake test formula was varied under different atmospheric pressures.[1] As a result of this work, an equation was submitted for calculating the amounts of cream of tartar and soda to be used with the A.A.C.C. formula for various altitudes. According to the equation, $y = -0.03A^2 - 0.25A + 6$, where y represents the cream of tartar in grams and A the altitude in thousands of feet.

At the request of this year's chairman of the Committee on Methods of Testing Cake Flour, a subcommittee was formed to test various cake flours by the A.A.C.C. formula and Barmore's equation for adjustments in cream of tartar and soda used.

[1] Cereal Chem. **16**: 145 (1939).

Four samples of flour were sent to collaborators located at altitudes varying from 50 to 5,000 feet above sea level. All were located either in the Rocky Mountain states or on the Pacific slope. This arrangement made it possible to cover the range of altitudes in which cake production occurs in the western part of the United States. Descriptions and analyses of the flours are shown in Table I. Amounts of

TABLE I

ANALYSIS OF FLOURS

Sample	Moisture	Ash	Protein	Description
	%	%	%	
A	12.8	.36	8.5	Pacific Northwest cake flour
B	13.5	.34	7.7	Midwestern cake flour
C	12.8	.39	7.7	Pacific Northwest cake flour
D	13.4	.34	6.9	Rocky Mountain States cake flour

cream of tartar and soda used for the different altitudes, respectively, are given in Table II.

TABLE II

AMOUNTS OF CREAM OF TARTER AND SODA USED

Altitude	Cream of tartar	Soda
feet	*g*	*g*
50	5.99	2.99
1005	5.72	2.85
1968	5.39	2.69
3492	4.76	2.38
4725	4.15	2.07
5000	4.00	2.00
Standard A.A.C.C.	6.00	3.00

TABLE III

COMPARISON OF TOTAL SCORES ON FLOUR SAMPLES A, B, C, AND D

Collaborator's altitude	Sample A		Sample B		Sample C		Sample D	
	Regular formula	Adjusted formula	Regular formula	Adjusted formula	Regular formula	Adjusted formula	Regular formula	Adjusted formula
feet								
50	83	82	90	90	64	65	72	72
1005	—	—	—	—	77	77	90	91
1968	83	85	90	91	84	90	91	92
3492	80	92	43	53	—	—	—	—
4725	95	93	74	87	65	63	82	84
5000	76	84	46	66	64	80	60	75
Average	83.4	87.2	68.6	77.4	70.8	75	79	82.8

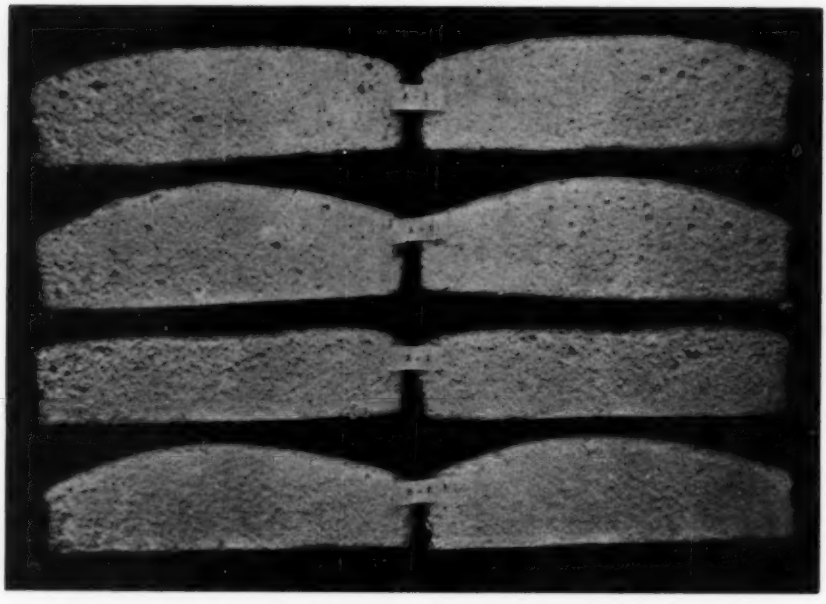

Fig. 1. Cakes from flours A and B, baked at 5,000 feet. A-1 = flour A, standard formula. A-2 = flour A, adjusted formula. B-1 = flour B, standard formula. B-2 = flour B, adjusted formula.

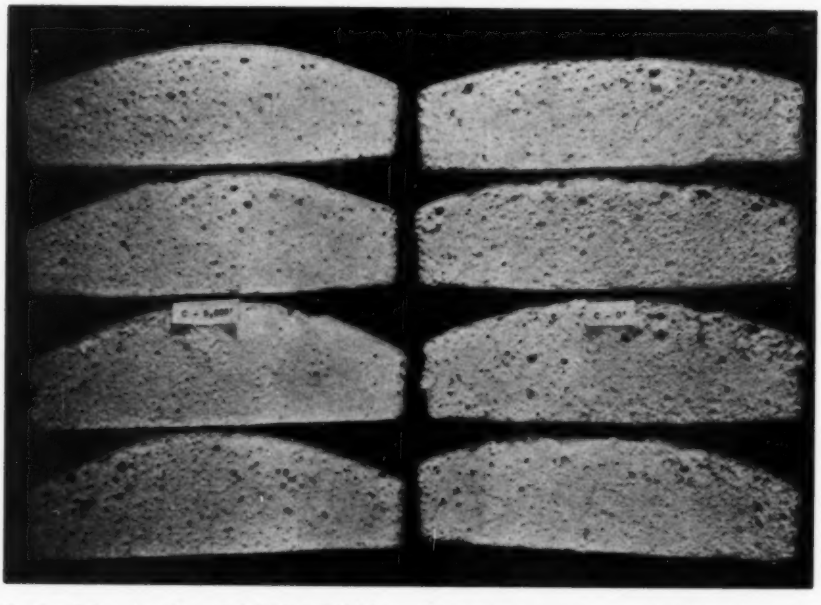

Fig. 2. Cakes from flour C, baked at 5,000 feet. RIGHT-HAND GROUP = standard formula. LEFT-HAND GROUP = adjusted formula.

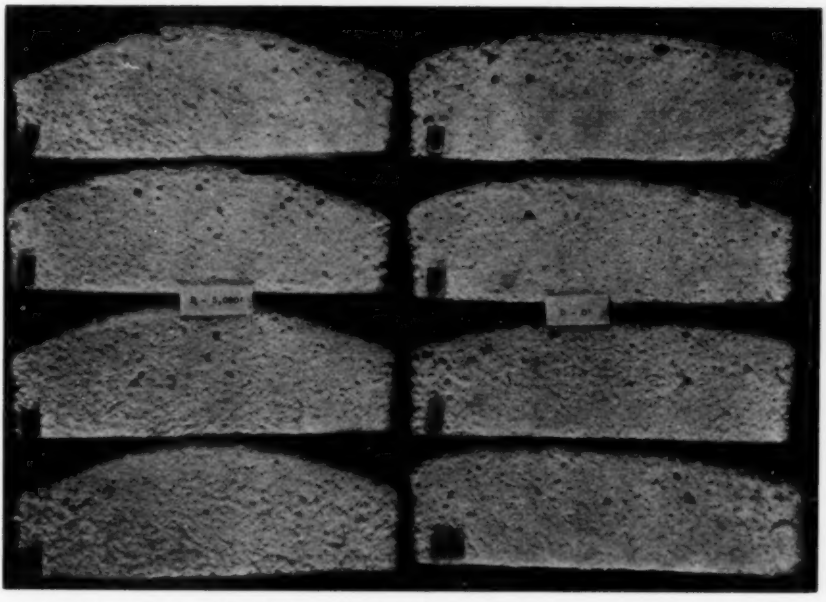

Fig. 3. Cakes from flour D, baked at 5,000 feet. RIGHT-HAND GROUP = standard formula. LEFT-HAND GROUP = adjusted formula.

All collaborators baked two series of cakes from each flour sample. In one series the bakes were made by the A.A.C.C. formula. In the other, the cream of tartar and soda were adjusted, by means of Barmore's equation, to the particular altitude involved.

Collaborators were requested to score the cakes as carefully as possible, by the A.A.C.C. scoring procedure, and to make comments on results obtained by using the specially adjusted quantities of baking powder. The scores reported on the bakes are recorded in Table III.

Figures 1, 2, and 3 are photographs of bakes conducted at the 5000-foot elevation.

From an inspection of Table III and of the figures, it is evident that the adjustments in the amount of leavening agent produced results that varied with the flour, on the one hand, and with the altitude on the other. It is of course impossible to properly judge the significance of these variations without knowing how closely the results would agree on many repeated bakes by the same operator, as well as by several operators working at each of the different altitudes. Flour B showed the greatest response to the adjusted formula, and it appears that some flours have more tolerance to baking conditions at different altitudes than others.

Aside from the collaborator who worked at sea level, it was the general opinion among collaborators that cakes baked with the adjusted

formula were somewhat better than those baked with the standard formula.

Some collaborators reported that future studies should include altitude adjustments for sugar as well as for baking powder, and one reported an observation that certain types of shortening behave differently, in a cake batter, at different altitudes.

Summary

A collaborative cake-baking study, involving adjustments in amount of baking powder to conform to different altitudes, was undertaken. In general the adjustments brought about an improvement in cake scores, although the nature and degree of response varied considerably among the different flours as well as among collaborators working at different altitudes.

There is need for further study, which might properly consider ingredients other than baking powder.

REPORT OF THE 1942–43 COMMITTEE ON METHODS OF TESTING CAKE FLOUR

LOWELL ARMSTRONG, Chairman

Ballard & Ballard Co., Louisville, Kentucky

(Read at the Annual Meeting, May 1943)

The committee work for this year dealt with the application of the tentative cake test to the study of a practical problem, namely, the effect of varying flour pH on the baking quality of flours of differing ages.

TABLE I

EFFECT OF FLOUR pH ON CAKE BAKING QUALITY AT DIFFERENT INTERVALS OF STORAGE

(Average of Collaborators' Scores and Data for A.A.C.C. Formula)

Flour	1st Baking Age of flour—7 days			2nd Baking Age of flour—30 days			3rd Baking Age of flour—71 days		
	pH of flour	Vol. of loaf	Score	pH of flour	Vol. of loaf	Score	pH of flour	Vol. of loaf	Score
A	5.36	772	77.2	5.30	874	78.6	5.23	834	80.0
B	5.04	762	83.8	5.02	856	84.6	4.93	824	83.8
C	4.76	752	86.7	4.73	799	85.3	4.67	833	82.6

An unbleached, commercially milled, soft wheat flour containing 8.1% protein and 0.35% ash on a 15% moisture basis was prepared from mill streams of known cake-flour performance, and chlorine treated in a

laboratory bleacher to pH levels of 4.7, 5.1, and 5.4. Sufficient Novadelox (3/4 oz per barrel) was added to each sample to give good color removal.

The three samples were submitted to six collaborators who baked them by the A. A. C. C. formula after storage for 7, 30, and 71 days respectively. A seventh member baked the samples after similar intervals of storage by a commercial-type formula. The collaborators who used the A. A. C. C. formula were asked to score the cakes by the official score card and the averages of the data they reported are given in Table I.

The member who employed the commercial-type formula reported similar data but gave the flours rankings instead of scores; his results are shown in Table II.

TABLE II

EFFECT OF FLOUR pH ON CAKE BAKING QUALITY AT DIFFERENT INTERVALS OF STORAGE

(Results with Commercial-type Formula)

Flour	1st Baking Age of flour—8 days			2nd Baking Age of flour—30 days			3rd Baking Age of flour—71 days		
	pH of flour	Vol. of layers[1]	Rank	pH of flour	Vol. of layers[1]	Rank	pH of flour	Vol. of layers[1]	Rank
A	5.53	1005	2	5.29	1130	1	5.30	1005	1
B	5.20	1025	1	4.94	1115	1	4.95	1000	1
C	4.91	985	3	4.68	1050	2	4.70	1010	1

[1] Weight of batter 400 grams.

The following conclusions have been drawn from the results of the committee's work—

1. A tendency toward a tight grain in cakes from flour of low pH value was demonstrated in both the A. A. C. C. formula and the commercial-type cake.

2. Closely knit, tighter-grained cakes are given a higher score where the official score card values are used.

3. Bakers and commercial men are inclined to reverse the ratings and give preference to the cakes with larger volumes.

4. Flours of high pH value improve in baking performance with age, while flours of low pH decline.

5. Confirming former reports, flours increase in acidity with age.

The members reported large variations in the cake volumes obtained in baking the same flour on different days. It is thought that these variations were caused by factors other than the flour itself. Therefore, the committee recommends that a project be planned and executed to study the factors affecting volume.

Acknowledgments

The members who participated in this year's work were Donald Wade, William Haley, W. E. Stokes, R. W. Mitchell, J. W. Montzheimer, W. V. Van Scoyck, and H. V. Moss.

REPORT OF 1942–43 METHODS OF ANALYSIS SUBCOMMITTEE ON THE DETERMINATION OF IRON IN CEREAL PRODUCTS

M. Howe, Chairman

Russell Miller Milling Co., Minneapolis, Minn.

(Read at the Annual Meeting, May 1943)

In a continuation of the work of the 1941–42 Subcommittee of the Committee on Methods of Analysis concerning the determination of iron in cereal products (Sullivan, 1943), four samples of cereals, together with a standard iron solution prepared from iron wire, were sent to 13 collaborators. These samples comprised: (1) an unenriched patent flour; (2) the same flour enriched with 5 mg Fe per lb as ferrum reductum; (3) the same flour enriched with 6 mg Fe per lb as sodium iron pyrophosphate; and (4) a whole wheat flour. As in the previous study, the collaborators were requested to analyze these samples by the α,α' dipyridyl method, essentially as outlined by Andrews and Felt (1941). Ashing of the samples at 550°–600°C and treatment of the ash with hydrochloric acid were specified; in addition, three of the collaborators also carried out determinations in which the alkaline fusion technique was employed in preparing solutions from the samples. The results submitted by the 10 collaborators who reported data by the specified method are recorded in Table I; the supplementary data obtained by the three collaborators who employed alkali fusion are shown in Table II.

A comparison of the summarized results given at the end of Table I with those reported last year (Sullivan, 1943), shows that the collaborators are in closer agreement this year but the variations are still quite large, particularly in the instance of the whole wheat flour. It was suggested that the whole wheat flour might have been contaminated with small pieces of metal from the milling rolls. This was confirmed by two of the collaborators who passed a magnet over a thin layer of the sample. In view of this observation, whole wheat flour and unenriched farina to be employed in future collaborative studies should be passed over a powerful electromagnet.

Two of the three collaborators who employed alkaline fusion in preparing the samples for analysis reported results in fair agreement

TABLE I

IRON CONTENT OF CEREAL PRODUCTS AS DETERMINED BY 10 COLLABORATORS

Collaborator no.	α,α′ dipyridyl method—ash treated with HCl. Iron content as Fe ("as is" moisture basis)			
	No. 1 Unenriched flour	No. 2 Flour enriched with 5 mg per lb Fe as ferrum reductum	No. 3 Flour enriched with 6 mg per lb Fe as sodium iron pyrophosphate	No. 4 Whole wheat flour
	mg/lb	*mg/lb*	*mg/lb*	*mg/lb*
1	3.6	8.6	10.0	16.3
2	3.9	8.8	9.9	16.1
3	4.3	8.4	9.5	18.5
5	4.3	9.1	9.2	19.3
6	3.5	8.5	7.9	24.8
8	3.5	7.3	9.3	15.6
9	3.3	8.5	10.5	19.4
11	3.7	8.7	10.0	17.0
12	4.1	10.0	12.7	15.4
13	3.6	8.6	9.8	16.2
Minimum	3.3	7.3	7.9	15.4
Maximum	4.3	10.0	12.7	24.8
Mean	3.8	8.6	9.9	17.9
Percentage of collaborators agreeing within:				
± 5% of mean	40	70	50	20
±10% of mean	70	80	80	70

with those obtained by acid treatment of the ash. The third collaborator obtained significantly higher results than the other two. It is interesting to note that his values for samples No. 2 and No. 3 exceed the iron content as calculated from the addition of 5 and 6 mg

TABLE II

IRON CONTENT OF CEREAL PRODUCTS AS DETERMINED BY THREE COLLABORATORS EMPLOYING ALKALINE FUSION OF ASH

Collaborator no.	Reagent	Iron content as Fe ("as is" moisture basis)			
		No. 1 Unenriched flour	No. 2 Flour enriched with 5 mg per lb Fe as ferrum reductum	No. 3 Flour enriched with 6 mg per lb Fe as sodium iron pyrophosphate	No. 4 Whole wheat flour
		mg/lb	*mg/lb*	*mg/lb*	*mg/lb*
8	α,α′ dipyridyl thiocyanate	3.5 3.6	8.4 9.1	9.5 9.6	16.0 16.9
3	ortho-phenanthroline	5.0	11.5	12.1	18.7
13	α,α′ dipyridyl	3.6	8.4	9.9	16.2

per lb Fe respectively; this indicates that the method was yielding erratic results in his hands.

From the comments of the collaborators and also from our own observations, the following precautions are suggested. The dipyridyl color is rather slow in developing and it is suggested that the samples should stand at least 30 minutes before the readings are made. A comment was received from one collaborator stating that the buffer was not strong enough to bring the pH to 4.0. The pH on a series of whole wheat flours using both hydrochloric acid and sodium carbonate was measured and found to vary from pH 4.06 to pH 4.16; these results would indicate that the buffer is satisfactory. It is again suggested, however, that the volume of the buffer should be increased to 10 ml to provide sufficient solution for certain types of colorimeters.

There still exists a great deal of confusion and doubt concerning the loss of iron during dry ashing. We have used wet digestion methods numerous times in our laboratory in comparison with dry ashing and could detect no loss of iron upon ashing of flours and wheat. This fact has been substantiated by several of the collaborators. The problem may be more complicated in the case of bread and similar baked products.

Many of the collaborators reported that the iron solution sent out with the samples gave a curve identical with that obtained for their own standard solution.

It is recommended that an exhaustive comparison be made on various samples of cereals including flour, whole wheat, bread, and farina, using both alkaline fusion and acid treatment of the ash for the determination of iron by the α,α' dipyridyl method. It is also suggested that comparisons be made between dry and wet ashing procedures.

Acknowledgments

The members who collaborated in this study were: J. S. Andrews, C. N. Frey, Chas. Hoffman, E. Hove, M. Howe, Morris Mead, Wendell Reeder, Oscar Skovholt, W. R. Urban, and Lawrence Zeleny.

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REPORT OF THE 1942–43 METHODS OF ANALYSIS SUBCOMMITTEE ON THIAMINE ASSAY

OSCAR SKOVHOLT, Chairman

Quality Bakers of America, New York, N. Y.

(Read at the Annual Meeting, May 1943)

During the year, 10 monthly samples have been distributed for thiamine determinations. The first sample in July was received by 29 collaborators and the list increased to 47 for the last sample. Of these, 38 used the thiochrome procedure and 9 employed the fermentation method. Most of the larger cereal laboratories in the United States and Canada were represented. Samples were distributed by volunteers among the collaborators and reports were compiled by the chairman. One of the collaborators distributed standardized thiamine solutions with the November sample and results obtained with this standard were compared with those from the solutions regularly used in each laboratory.

Attempts were made to obtain complete records of the technique and procedure used in each laboratory. Certain facts were obtained with regard to most details of procedure in many laboratories. Complete records were not obtained of the many variations that have apparently been adopted in some laboratories. A suggestion for more detailed study of these factors is made later in the report.

Results of the year's activities were far from satisfactory, although it is believed that progress was made in some respects. Some collaborators used their relative standing in the monthly lists as a guide to determine the need for careful scrutiny of their own procedures. It is hoped that all collaborators will make such a careful study of the summarized record as set forth in Table I. This table is a compilation of all reported results expressed only as the deviation from the average value obtained for that sample by each method. At the right-hand side of the table is found the relative number of samples on which each collaborator reported values that were above and below the mean. The figures do not include the few cases in which the reported value exactly equaled the average.

Since reliable replicated results by bio-assay are not available, the average values by the procedures used must be considered as the best available measure of the actual thiamine content. Emphasis is placed on this matter of average value, since it is recognized that each collaborator who quite consistently tends to be high or low may be convinced that the average is not correct. It may be stated that several laboratories which have extensively studied vitamin-testing methods

and whose reputation for general accuracy is of the highest order, are included either among those that consistently report well below the mean or among those that are equally above the average value. It is believed that the method of compiling the results will bring out this

TABLE I

COLLABORATORS' THIAMINE RESULTS EXPRESSED AS DEVIATIONS FROM THE AVERAGE

(In gamma per gram on as-received moisture basis)

Coll. no.	Wheat fractions		Enriched bread			Enriched flour				No. of times	
	Cereal	Germ									
	July	Jan.	Sept.	Oct.	Dec.	Nov.[1]	Feb.[3]	Mar.[3]	April[4]	Above mean	Below mean
					THIOCHROME METHOD						
1	− .36	+ .10	− .29	− .46	− .17	− .23	− .08	+ .22	− .11	2	7
2		− .20			− .36	+ .14	− .57	+ .12	− .22	2	4
3		− 1.30		− .12	− .52	− .42	− .43	− .55	− .17	0	7
4		+ .10	− .09	+ .29	− .03	− .36	− .11	− .27	− .89	2	6
5			+ .46			+ .14				2	0
6	+ .54	+ 1.80	− .03	+ .33	+ .51	+ .73	+ .40	+ .73	+ .89	8	1
7	+ .01		− .26	− .74	+ .20	+ .22	− .27	− .09	.00	3	4
8	− .15	+ .80	− .09	− .36	+ .13	− .18	− .08	− .01	− .11	2	7
9		− 1.40					− .22	− .41	− .80	0	4
10	− .14	− 2.50	+ .03	+ .29	+ .99	+ .59	− .12	− .02	− .11	4	5
11	+ .03	− 1.70	+ .65	+ .08	− .36	− .20	− .35	+ .16	− .03	4	5
12	− .53	− 2.90	+ 1.17	+ 2.53	+ .81	+ .04	+ .23	.00	− .24	5	3
13	− .52	− 3.40	− .04	+ .31	− .52	− .06	− .03	− .51		1	7
14		− .60		− .61	− .30	− .29	+ .22	+ .04	− .04	2	5
15		− 1.30	− .12	− .01	− .31	− .24	+ .29	+ .09	− .13	2	6
16		+ 9.60		− .18	+ .08	+ 1.28	+ .46	− .01	− .10	4	3
17	+ .11	+ .60		+ .35	+ .30	− .22	− .25	− .29	− .41	4	4
18	− .18	− 2.70	− .18	+ .02	− .15	+ .03	− .20	− .04	− .11	2	7
19						− .43				0	1
20		− 2.30					+ .19			1	1
21		− 2.50	− .28	+ .28	− .37	− .19	+ .53	+ .34	+ .75	4	4
22	+ .16	+ .30	+ .17	− .03	+ .24	+ .06	+ .15	− .28	− .05	6	3
23		− .60	− .93				− .14		− .33	0	4
24								− .17	+ .04	1	1
25	+ .52	+ 2.70		+ .42	+ .36	+ 1.17	− .39	− .01	− .61	5	3
26	− .07	− 1.60	− .13	− .31	− .23	− .50				0	6
27	− .37	+ .30	+ .34	+ .54	+ .09	− .10	+ .02	+ .02	+ .08	7	2
28	− .40	+ .80	− .27	− .40	− .43	− .02	− .10	+ .34	− .45	2	7
29		+ 4.10			+ .30	+ .21	+ .25	− .03	+ .71	5	1
30	− .17	− .10	− .32	− .08	− .02	− .29	− .03	+ .02	− .07	1	8
31	− .20	+ 4.20	+ .26	− .70	+ .13	− .15	− .14	− .06	+ .52	4	5
32	+ .01	+ 1.00	+ .09	+ .13		− .07	+ .27	− .16	+ .59	6	2
33	− .15	− 2.90	+ .02	− .05	− .02	− .14	− .04	− .20	+ .20	2	7
34	+ .72	− 1.30	+ .13	− .02	+ .41	− .16	+ .16	+ .14	+ .16	6	3
35	+ 1.10	− .70	+ .32	− .51	− .54	.00	+ .34	+ .51	+ .36	5	3
36		+ .70	− .28	− .31	− .23	− .19	+ .11	+ .09	+ .25	4	4
37	− .02	+ 2.90	− .27	− .65	− .11	− .26	− .15	+ .39	+ .20	3	6
47									+ .03	1	0
Mean	2.67	26.50	2.58	3.52	3.37	3.86	3.85	4.04	5.37		

TABLE I—(*Continued*)

Coll. no.	Wheat fractions		Enriched bread			Enriched flour				No. of times	
	Cereal	Germ									
	July	Jan.	Sept.	Oct.	Dec.	Nov.[1]	Feb.[2]	Mar.[3]	April[4]	Above mean	Below mean
					FERMENTATION METHOD						
38	+ .69		+ .33	− .48						2	1
39	− .42	− 2.00	− .06	+ .04	+ .06	− .28	− .14	− .18	− .66	2	7
40		− 1.70			− .04	+ .02				1	2
41	+ .23	+ 3.20	+ .13	+ .37	+ .78	− .36	− .06	− .12	+ .47	6	3
42		− .40	− .14	− .12		+ .17		− .08	+ .15	2	4
43	− .19	+ .70	− .33	− .24	− .03	− .38	+ .09	− .01	+ .50	3	6
44	+ .13	+ 2.30	+ .53	+ .45	+ .13	+ .20	− .02	− .04	+ .32	7	2
45	− .52	− .90		− .04		+ .76		+ .01	− 1.05	2	4
46	+ .06	− .90	− .44	− .84	− .88	− .16	+ .11	+ .44	+ .24	4	5
Mean	2.72	27.60	2.54	3.63	3.22	3.96	3.99	4.12	5.46		

[1] Values obtained on ordinary enriched flour using distributed sample of thiamine solution for reference.
[2] Ordinary enriched flour.
[3] Phosphated enriched flour.
[4] This sample contained riboflavin, thiamine, niacin, and iron at the newly-proposed higher levels for enriched flour.

fact. It seems advisable for each collaborator to note in particular how many times the reported results from his or her laboratory were above or below the mean and the magnitude of the differences. It is believed that next year's committee should carefully compare the procedures in use in a few of those laboratories that were consistently high with those that were low, in attempting to determine the reason for such consistent differences.

Many collaborators show no consistency as regards relationship to average value. This may indicate the need for further standardization of technique in many cases. It seems that precision in every operation is essential to obtain reproducible results. It should be pointed out, however, that replicate results as occasionally reported by collaborators show much less variation than is evident when comparing averages between laboratories. Another reason for a lack of consistency throughout the year in the results of some collaborators when compared with the mean, is the fact that improvement in technique was stimulated by the monthly reports. Sources of error were found which were corrected in later determinations.

It would be pleasant to be able to report that such stimulation caused a marked increase in accuracy during the course of the year, but the progress made was relatively minor, or it might even be debated whether any improvement was shown. Monthly reports have

indicated the range of results by each method and have shown the number of collaborators agreeing within plus or minus 5% and 10% of the mean value. A better measure of degree of agreement is undoubtedly obtained by statistical analysis. Coefficients of variability have been calculated for results on nine of the samples distributed during the year. It should be mentioned that one sample of yeast distributed in August caused considerable difficulty in most laboratories, owing to lack of experience with such material. One collaborator with considerable experience in the assay of such material mentioned that the character of this sample was far more variable than any yeast previously examined. Results reported were so widely variable that it seems inadvisable to include them in this report.

The extent of agreement of results, as indicated by coefficients of variability, on the other nine samples as obtained by thiochrome and fermentation methods will be found in Table II. The samples are in

TABLE II

AN ANALYSIS OF THE PRECISION OF THIAMINE ASSAYS

	Wheat fractions		Enriched bread			Enriched flour			
	Cereal	Germ							
	July	Jan.	Sept.	Oct.	Dec.	Nov.[1]	Feb.[2]	Mar.[3]	April[4]
THIOCHROME METHOD									
No. of Coll.	22	33	28	29	30	33	33	32	33
Mean Thiamine, *ug/g*	2.67	26.5	2.58	3.52	3.37	3.86	3.85	4.04	5.37
Coeff. of Var. %	15.2	9.7	14.6	17.2	11.4	10.6	7.0	6.9	7.6
FERMENTATION METHOD									
No. of Coll.	7	8	7	7	6	8	5	6	7
Mean Thiamine, *ug/g*	2.72	27.6	2.54	3.63	3.22	3.96	3.99	4.12	5.46
Coeff. of Var. %	15.2	6.8	13.8	12.1	16.5	9.7	2.6	5.0	11.1

[1] Values obtained on ordinary enriched flour using distributed sample of thiamine solution for reference.
[2] Ordinary enriched flour.
[3] Phosphated enriched flour.
[4] This sample contained riboflavin, thiamine, niacin, and iron at the newly-proposed higher levels for enriched flour.

three groups, namely, wheat fractions, enriched bread, and enriched flour. The wheat fractions group includes a cereal and a wheat germ sample. Three samples of enriched bread are included and four samples of enriched flour. The March sample was a phosphated enriched flour and the April sample contained riboflavin, thiamine, niacin, and iron at the newly-proposed, higher levels. The table also

includes data on number of collaborators reporting in each case and the average value as obtained.

The latter figure is included primarily since another contribution at this convention[1] will show that coefficients of variability are usually lowest when the sample analyzed is high in thiamine potency. This is probably true when dealing with similar materials, but in the case of this series, it seems evident that type of sample tested is most important.

The relatively high coefficient for the cereal sample may partly be due to the fact that it was the first in the series. The enriched bread samples average higher in variability than the flours. It has been previously pointed out that this may be due to unsolved extraction problems. The last of this series produced results with least variability by the thiochrome method but with greater error than on the other two samples when using the fermentation procedure. Some improvement in precision was shown on enriched flours when using the thiochrome method except in the case of the last sample. The slightly increased coefficient in this case was probably accidental and may have no relationship to the inclusion of riboflavin and higher levels of other factors. It is not known whether the sharp increase in variability when using the fermentation method on this material as compared with the excellent agreement shown on February and March samples indicates some disturbance of other materials added to the flour, but this does not appear to be likely as judged by the agreement between this mean value and that for the thiochrome method. The January wheat germ sample was analyzed with fair accuracy but no better than the average shown on flour samples in spite of the higher potency of this material. It is evident that the mean values and coefficients of variability are quite similar for the thiochrome and fermentation methods. Considering the entire series the average coefficient of variation for the thiochrome method is 11.1% as compared with 10.3% for the fermentation procedure.

No listing is made of results obtained when collaborators' own standard solutions were used in testing the November sample. The values given are those obtained when using the distributed standardized thiamine. The effect of this uniform standard was relatively small. Nearly all collaborators found their own solutions to be similar to the standard in concentration. The coefficient of variation for the thiochrome method was 11.4% when using their own solutions as compared with 10.6% for the standardized thiamine.

The strength of thiamine standard is a factor in variability but

[1] Hildebrand, F. C., and Geddes, W. F. The experimental error of the thiochrome method for thiamine assay. (To be published in a subsequent issue of this Journal.)

other sources of error may be more important. Careful timing of all operations in the later stages is essential when using the thiochrome method. With some bread samples in particular, it seems that type of enzyme and pH of medium are important in obtaining full extraction. This seems to be more critical in certain laboratories than in others for some unknown reason. The question of optimum ferricyanide level as it applies to the standards and the sample has not been settled. It seems evident that excess of ferricyanide lowers galvanometer readings, but the question remains as to whether or not this cancels out when using the same fixed amounts for the standard and the unknown. Some evidence indicates that the amount recommended in published procedures causes more of an excess and a greater lowering of readings with the standard than when introduced into samples of certain materials. It seems that more studies should be made to determine the minimum amount needed to produce maximum fluorescence in the standards and in samples of various materials. In the fermentation method, the large variability in sulphite blanks is difficult to explain. The blanks have not been listed in this summary but on most samples the highest blank was as much as 4 or 5 times the lowest one reported.

These suggestions are made with no pretense at covering all critical steps in thiamine determinations. Actual studies of procedure can best be made in one or only a few cooperating laboratories. It is felt that the data obtained may serve as a guide in selecting laboratories for a careful comparison of procedures. Such comparisons cannot be thoroughly enough made by merely comparing written details of method. It may be necessary to watch every move by two different analysts to determine why their results are consistently different. It is suggested that collaborators with high and low averages might profitably visit each other to observe methods in use, if this is necessary to uncover the reason for differences. The person assigned to the task of studying methods and uncovering the reason for consistent differences should be one well versed in the actual determination and one whose major interest is in thiamine assay so that all literature on the subject is closely followed. Your present chairman does not measure up to these requirements.

The specific recommendation made at present is to have someone assigned to this critical comparison of thiamine assay methods in a limited number of laboratories. It is difficult to make recommendations on the subject of continuing monthly check samples as in the past year, and if this is done it should be supplementary to an intensive study of methods. There is a real demand for such service and it may serve a useful purpose. Possibly some simplified system for

handling samples and reports could be adopted in order to reduce the burden of this assignment. Some fee might be charged to cover expenses or it might become an activity of certain local sections. It is advisable to give consideration to the use of any check-sample system for determinations of all vitamin and mineral factors of current interest in cereals instead of limiting the checking to thiamine only. This would have particular advantages if proposed methods for the use of the same extract in the determination of several vitamins should prove practical. The matter of the future of such collaborative checking is left in the hands of the association officials without definite recommendation, although further specific studies of methods in use is urged as highly desirable.

REPORT OF 1942–43 METHODS OF ANALYSIS SUBCOMMITTEE ON RIBOFLAVIN ASSAY [1]

JOHN S. ANDREWS, Chairman

General Mills, Inc., Research Department, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1943)

This report deals with a continuation of collaborative studies of riboflavin assay methods inaugurated a year ago. Its purpose is to evaluate further the procedures designed for the determination of riboflavin in cereal products. In planning this continued study, attention has been focused primarily upon the problems presented by the expectancy that riboflavin will shortly become one of the required ingredients of enriched flour.

Early this year, letters were sent to some 35 qualified laboratories requesting their cooperation in studying riboflavin assay procedures. Thirty-one of these laboratories indicated their desire to participate in the program. Most of them indicated their willingness to test the submitted procedure, and all agreed to submit assays of the cereal samples.

In order to simplify this collaborative work as far as possible, only one method was presented. This was a fluorometric procedure similar to that employed in the committee's earlier activities (Andrews, 1943). Selection of a fluorometric rather than a microbiological procedure was made since the speed of the former makes it more adaptable to the requirements of control assays.

In presenting this method the collaborators were asked to observe particularly two factors which appeared to have an important bearing upon the results. One of these pertained to the use of acid extraction

[1] Paper No. 49, Journal Series, General Mills, Inc., Research Department.

to liberate any combined forms of riboflavin. The second involved the question of sample size. Work carried out in the writer's laboratory has suggested that the quantity of sample taken for assay has a significant bearing on the results, the values decreasing with increasing size. For this reason, small samples were recommended, and wherever concentration of the extracts was required it was suggested that larger aliquots be passed through the "florisil." Details of the collaborative procedure are presented below:

FLUOROMETRIC PROCEDURE FOR RIBOFLAVIN ASSAY

REAGENTS:

(1) Sulfuric acid solution, 0.1*N*
(2) Trisodium phosphate solution, 6.5% $Na_3PO_4 \cdot 12H_2O$
(3) Potassium permanganate solution, 4% (freshly prepared)
(4) Hydrogen peroxide solution, 3%
(5) Florisil, 60–100 mesh (Floridin Co., Warren, Pa.)
(6) Pyridine acetic acid solution, 20% pyridine in 2% acetic acid solution
(7) Sodium fluorescein stock solution, 10 mg/1000 ml. (10 μg/ml)
(8) Sodium fluorescein dilute solution, 0.1 μg/ml
(9) Riboflavin standard solution, 1.4 μg/ml in pyridine acetic acid solution (reagent 5)
(10) Acetic acid, glacial

SPECIAL APPARATUS:

(1) Adsorption columns; overall length 160 mm; bottom constriction, 2 mm inside diameter ×15 mm long; column, 10 mm inside diameter ×95 mm long; bell-shaped top, 40 mm inside diameter ×50 mm long.
(2) Fluorophotometer
(3) Cylinders, glass stoppered, graduated to 25 ml

SAMPLE SIZES:

Whole wheat flour	1.5 g
Patent flour	5 g
Fortified patent flour	1 g
Fortified white bread	1 g

PROCEDURE:

Suspend the above quantity of sample in 50 ml 0.1*N* H_2SO_4 and autoclave at 15 lb pressure for 15 minutes. Cool. Add 12 ml of 6.5% Na_3PO_4 solution and 2 ml of glacial acetic acid. Dilute to 100 ml with distilled water and filter through No. 1 Whatman filter paper discarding the first few ml of filtrate.

To an aliquot (75 ml for patent and whole wheat flours, 50 ml for fortified flour and bread) of extract containing between 1 and 3 μg of riboflavin add 1 ml of permanganate solution, allow to stand 1 minute and add 1 ml of 3% H_2O_2 solution or enough to decolorize.

Prepare the adsorption column by first placing a wisp of cotton in the bottom and then adding florisil to a height of about 1 to 1½ inches. Wash once with 5–10 ml of distilled water.

Add the decolorized extract to the column and allow it to flow through by gravity. Wash the florisil twice with 5–10 ml portions of distilled water and then dry by drawing air through for a few seconds.

Elute the adsorbed riboflavin by passing 20 ml of pyridine acetic acid solution (6) through the column. Collect the eluate in the 25 ml graduated cylinder and make up to 20 ml with the pyridine acetic acid solution. Mix thoroughly.

Standardize the fluorophotometer by setting the galvanometer scale to zero with the pyridine acetic acid solution (6) in the cuvette. Next set the galvanometer deflection for 25 [2] by means of the diaphragm using the dilute fluorescein standard (8).[3]

[2] Applies to Pfaltz and Bauer instrument.
[3] Fluorescein varies in purity; in some instances only half this concentration is required to give a deflection of ca 25 when the diaphragm is one-half to two-thirds open.

Place 14 ml of the eluate in the cuvette and note the deflection (A). Add 1 ml of the riboflavin standard to the unknown in the cuvette and again note the deflection (B). Stir a few grains [4] of sodium hydrosulfite into the unknown in the cuvette and observe the deflection (C). Just before making each reading check the light intensity with the dilute fluorescein standard and adjust the diaphragm to give a galvanometer reading of 25.

CALCULATION:

$$\text{Riboflavin, } \mu g/g\ ^{5} = \frac{A - 1.07\,C}{1.07\,B - A} \times 0.1 \times EF \times DF$$

Four samples of cereal products were submitted. These were whole wheat flour, patent flour, patent flour to which had been added 2.64 μg of pure riboflavin per g, and bread made from this enriched flour. The bread sample contained riboflavin supplied by the enriched flour, and in addition, approximately 0.4 μg/g of riboflavin (air-dried basis) derived from 2% of yeast.

Results are now available from 18 of the collaborators. Thirteen of the participating laboratories employed the fluorometric procedure described by the committee and in addition some of them used other fluorometric methods. Eight laboratories reported microbiological values derived from several modifications of the original Snell and Strong (1939) procedure.

Discussion of Results

Figure 1 reveals the assay values reported for the sample of patent flour by both fluorometric and microbiological methods. It will be noted that the average obtained by the fluorometric procedure is considerably below that obtained microbiologically (that is, 0.32 as against 0.50 μg/g). This tendency on the part of the microbiological method to produce higher values was observed throughout the collaborative study. The histogram reveals a rather wide scattering on the part of the individual assays, since the values ranged from a low of 0.1 to a high of nearly 0.9 μg/g. While in such a product as patent flour the low riboflavin content could be expected to present difficulties for accurate assays, the present discrepancies must be considered excessive. Because of the known tendency of the microbiological method to produce high results, due to difficulties in removing nonflavin growth-promoting substances, it is believed that the average of the fluorometric assay in this instance is closer to the true riboflavin value. If we omit the two microbiological values which fall between 0.7 and 0.9, the microbiological average is slightly less than 0.4 μg/g, a figure in much closer agreement with that obtained fluorometrically. On the other

[4] Just sufficient to destroy the greenish-yellow fluorescence.

[5] 1.07 is the correction factor necessitated by the increase in volume caused by adding the standard. EF is the elution factor or ratio between the final volume of eluate and volume of extract passed through the column. DF is the dilution factor or ratio of the total volume of the extract to the weight of the sample.

hand, the largest number of fluorometric values fell within the range of 0.1 to 0.2 μg/g, and while these values are believed to be excessively low, there are no adequate reasons to substantiate this belief.

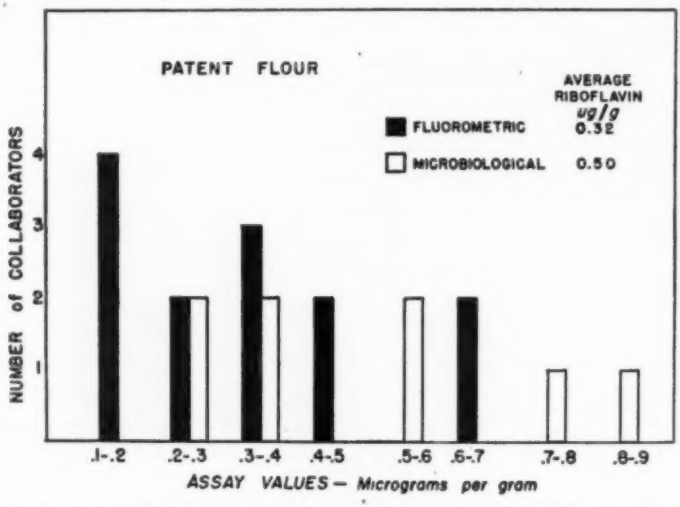

Fig. 1. Distribution of collaborators' riboflavin values for unenriched patent flour as determined by fluorometric and microbiological methods.

Figure 2 represents the collaborators' values obtained when pure riboflavin was added to the patent flour. Once again, the microbiological value (3.15) exceeds the fluorometric (2.62 μg/g). This discrepancy amounts to about 20%. On the other hand, there is much

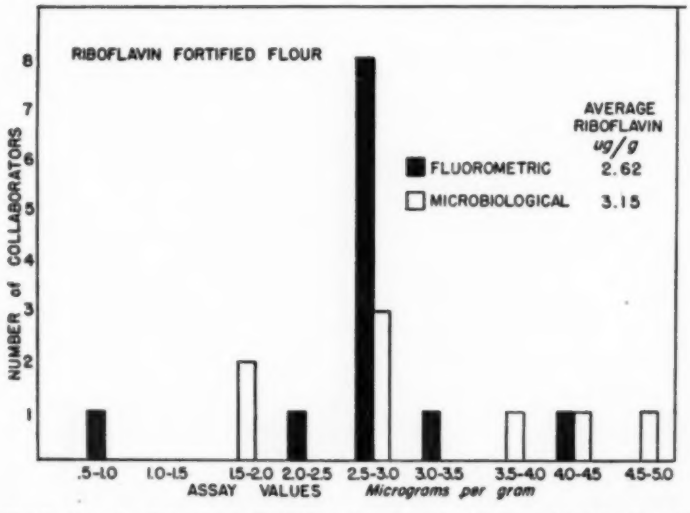

Fig. 2. Distribution of collaborators' riboflavin values for patent flour enriched by the addition of 2.64 μg pure riboflavin per g.

better grouping of the fluorometric values since the majority of these assays fell within the range of 2.5 to 3 μg/g. The microbiological values tended to be more widely scattered and once again tended toward higher figures.

By studying the assays of each collaborator for the patent and fortified flours it is possible to evaluate the results on the basis of the recoveries of the added riboflavin. These recovery data are shown in Figure 3. It will be noted that the average recovery in percent defi-

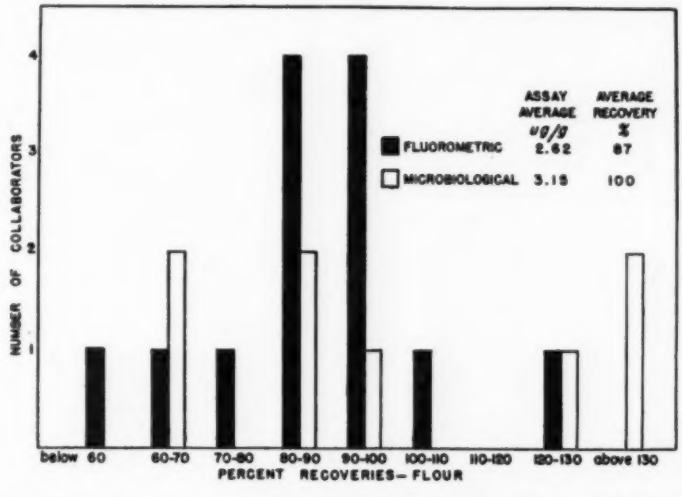

Fig. 3. Distribution of percentage recoveries of riboflavin added to patent flour obtained by different collaborators employing fluorometric and microbiological methods.

nitely favors the microbiological procedure, since it is quantitative, while in the instance of the fluorometric method the recovery is only 87%. One must be extremely cautious, however, in drawing significant conclusions from these observations. A study of the distribution of the collaborators' results reveals considerably greater disagreement in the instance of the microbiological method. As in the instance of the patent flour, two excessively high microbiological values accounted largely for the excellent average recovery. For this reason, it is believed that the microbiological findings must be regarded as entirely fortuitous until better agreement between laboratories can be achieved. Despite the fact that the recoveries obtained fluorometrically are somewhat low, the better distribution of individual values would appear to favor this type of procedure.

The results of an examination of another sample of flour containing added riboflavin was afforded by the April check sample of the Subcommittee on Thiamine Assay. Figure 4 presents the assay values

reported by 14 collaborators using fluorometric procedures. While the degree of unanimity between these 14 collaborators fails to reach the desired goal, it is perhaps encouraging to note the absence of widely divergent assays. This is particularly true when one considers that 50% of the values fell within a range of ±5%, and practically two-thirds of the collaborators agreed with ±10%.

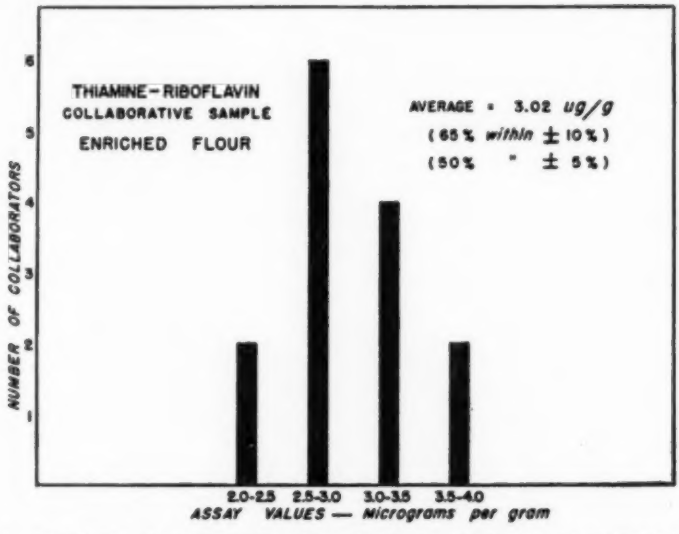

Fig. 4. Distribution of riboflavin values obtained by 14 collaborators using fluorometric procedures in assaying a sample of patent flour enriched with thiamine and riboflavin (April check test sample of Subcommittee on Thiamine Assay). Quantity of riboflavin added was 2.64 μg pure riboflavin per g.

The fluorometric methods employed by these collaborators were quite varied and incompletely described. They include the fluorometric procedure employed in last year's collaborative study, other modifications of the Conner-Straub (1941) procedure, and several so-called "chemical methods" developed in individual laboratories. This heterogeneity has prevented any accurate evaluation of the several fluorometric techniques represented. One of the future problems of the collaborative committee will be the evaluation of the various fluorometric procedures and standardization of the one which promises to be the most satisfactory.

Dr. Visser't Hooft, who prepared these samples, informs me that they were made by adding 2.64 μg/g of pure riboflavin. If we assume that the base flour originally contained between 0.3 and 0.4 μg/g, it will be noted that the average of 3.02 μg/g represents a very satisfactory recovery.

Figure 5 shows the results obtained from the assays of the fortified bread. The average obtained microbiologically exceeds the fluoro-

metric by approximately 15%. The tendency toward better grouping on the part of the fluorometric values would appear to favor this procedure, at least from the standpoint of reproducibility in different laboratories. However, there is considerable spread in both procedures, and cognizance should be taken of the fact that the number of microbiological values is considerably less than of the fluorometric.

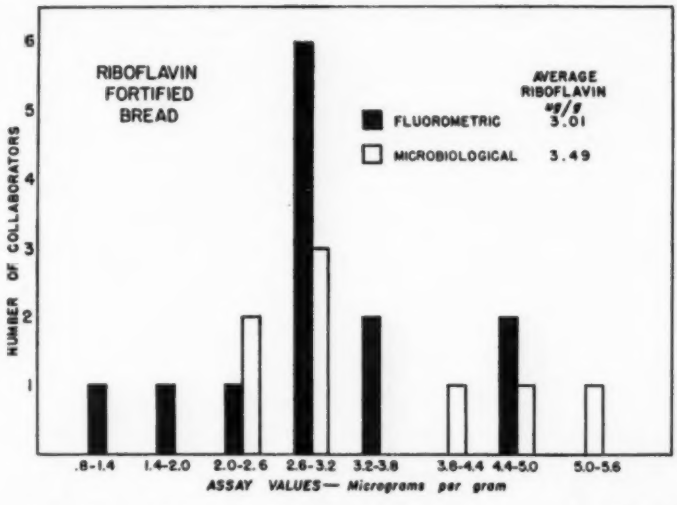

Fig. 5. Distribution of collaborators' riboflavin values for bread made from patent flour (enriched with 2.64 µg of pure riboflavin per g and approximately 0.4 µg riboflavin per g derived from yeast), as determined by fluorometric and microbiological methods.

Figure 6 reveals the percentage of recoveries based on the amount of riboflavin added to the patent flour both as crystalline material and in the form of yeast. In presenting these recovery values, it has been assumed that no measurable loss of riboflavin occurred during baking. Once again it will be noted that the best recovery was obtained in the instance of the microbiological method, since 98% of the added riboflavin was registered by the assay average. The recovery by the fluorometric procedure was essentially the same as that observed in the fortified flour. A study of the distribution of the assay values suggests that, on the average, bread offers greater difficulties. The fairly favorable average recovery appears to be attributable to a fortuitous balance between the excessively high and excessively low values from several individual laboratories. The available data do not reveal the cause of this discrepancy.

The wide divergence in the instance of the microbiological values and the distribution of these values is far from satisfactory. In the instance of the high microbiological values shown here, two of the three participating laboratories also employed fluorometric procedures.

One of these obtained excellent agreement between the fluorometric and microbiological assays, while the other reported fluorometric values which were much lower and closer to the fluorometric average.

A study of the assays reported by the individual laboratories reveals that in most instances there is a tendency to be consistent in all of the samples. Laboratories which appear to be low in the instance of the fluorometric determinations should give attention to the step employing adsorption on "florisil." A recent experience suggests that this operation may be one possible source of error. The case at point concerns an analyst who was obtaining quite satisfactory fluorometric assays, as based on recoveries, and who for some unknown reason suddenly began to experience extremely low values. Exami-

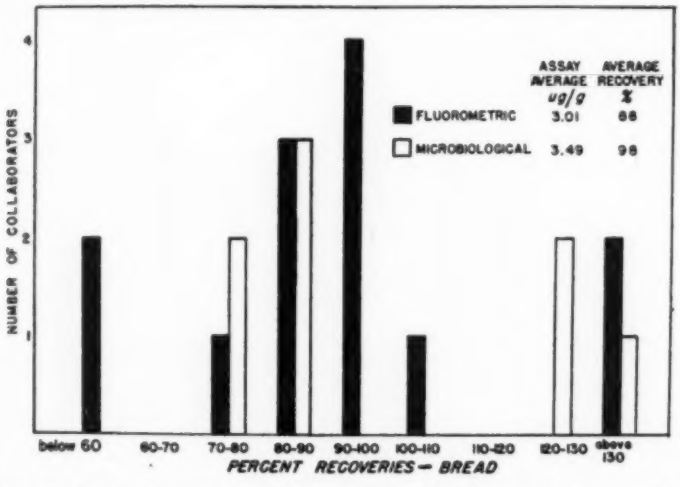

Fig. 6. Distribution of percentage recoveries of riboflavin in the analysis of bread made from enriched patent flour. Recoveries are based on the amount of riboflavin added to the patent flour both as crystalline material and in the form of yeast, assuming no loss during baking.

nation of the problem revealed that, at this point, he had changed from an old batch of "florisil" to a new supply. When tested with pure riboflavin the new lot of adsorbent gave practically quantitative assays, but when added to cereals the quantitative aspects were entirely lost. Upon returning to some of the initially used "florisil," no difficulty was experienced. It would thus appear that the difficulty could be attributed to variations in "florisil," and for this reason it is recommended that those analysts who experience low recovery values carefully examine this adsorption step. This examination should include not only the use of a pure riboflavin solution, but also a cereal extract to which known quantities of riboflavin have been added. In this way the question of the efficiency of the "florisil" adsorption can be established.

In the present collaborative study, there has been some indication that this same situation may prevail in other laboratories. Two of the collaborators carried out recovery experiments in addition to those represented in the samples and reported exceedingly low values. One collaborator was inclined to attribute this to the effect of heating, since when using a boiling water bath for the extraction he obtained much higher recoveries than with the autoclave. While the heating may have had some indirect effect on the adsorption, it is entirely possible that the quality of the "florisil" itself was not entirely adequate.

Figure 7 shows the collaborators' data for the sample of whole wheat flour. Once again the average microbiological value is considerably

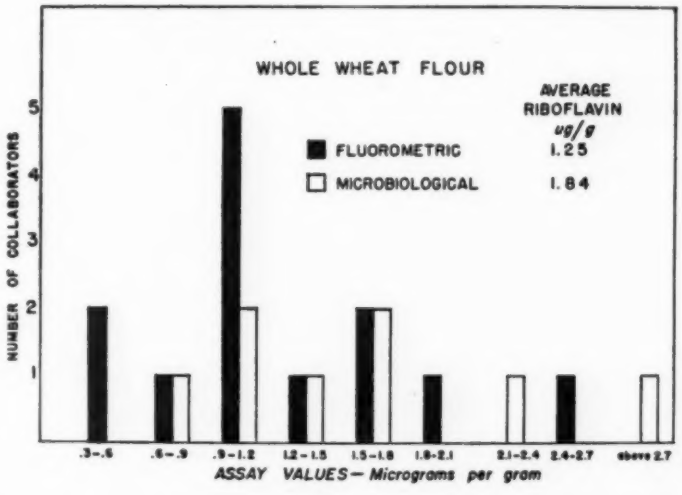

Fig. 7. Distribution of collaborators' riboflavin values for whole wheat flour, as determined by fluorometric and microbiological methods.

higher than the fluorometric. The groups of the individual values show better distribution for those measured by the fluorometric procedure. Two outstandingly high microbiological values account for practically all the discrepancy between the two types of assay procedures. It should be noted that this tendency toward high microbiological values is not entirely universal, since several collaborators obtained assays which must be considered too low.

Some of the collaborators carried out a fairly extensive study of their observations, and have made definite contributions in the way of comments and suggestions. One difficulty which was observed by several laboratories concerned the clarity of the extracts. The sample of bread, in particular, appeared to filter with considerable difficulty

and produced cloudy filtrates. It is suggested that those who encounter such difficulties should pay particular attention to the acidity of their extracts, since pH control will go a long way toward alleviating this difficulty.

There is still some question about the quantity of hydrosulfite which should be employed for reducing the riboflavin. There seems to be no question about the necessity of using limited quantities, since excessive amounts have an adverse effect. One collaborator recom-

TABLE I

SUMMARY OF COLLABORATIVE RIBOFLAVIN ASSAYS BY THE FLUOROMETRIC METHOD

Collaborator	Riboflavin in μg/g				Recovery of added riboflavin			
	Patent flour	Fortified flour	Fortified bread	Whole wheat flour	Flour		Bread	
					μg/g	%	μg/g	%
A	0.32	2.86	3.05	1.13	2.54	96	2.73	90
B	0.34	2.60	2.96	1.07	2.26	85	2.62	86
C	0.43	3.01	3.54	1.61	2.58	98	3.11	102
D	0.34	2.59	3.13	1.50	2.25	85	2.79	92
E	0.44	2.72	3.23	1.59	2.28	87	2.79	92
F	0.68	3.50	4.90	2.10	2.82	107	4.22	139
G	0.26	2.61	3.12	1.18	2.35	89	2.86	94
H	0.11	2.57	1.79	1.10	2.46	93	1.69	56
I	0.20	2.69	2.88	0.96	2.49	94	2.68	86
J	0.18	2.20	2.71	0.69	2.02	77	2.53	83
K	0.13	0.81	0.84	0.36	0.68	26	0.71	23
L	0.10	1.85	2.32	0.43	1.75	66	2.22	73
M	0.70	4.06	4.75	2.49	3.36	127	4.05	133
Mean	0.32	2.62	3.01	1.25	2.30	87	2.69	88
Minimum	0.10	0.81	0.84	0.36				
Maximum	0.70	4.06	4.90	2.49				
The following results were obtained too late to be included in the tabulation on which the graphs were based								
R	0.13	2.29	2.61	0.74	2.16	82	2.48	82

mends dissolving the hydrosulfite in pyridine and using two drops of this fresh solution (5%) in place of the solid.

Several collaborators confirmed the writer's observations that increased assay values accompanied the use of smaller samples and that acid extraction gave somewhat higher values than those obtained by extraction with water.

One collaborator suggested that the photosensitive nature of riboflavin would result in a loss between the initial reading and that obtained after adding pure riboflavin. Any prolonged exposure of the solution to the incident light should, of course, be avoided, and with proper precautions this does not seem to be a serious problem.

Several collaborators reported that bubbles resulting from decomposition of hydrogen peroxide interfered during the adsorption. This can be considerably eliminated by allowing the extract to stand 15 to 20 minutes prior to the adsorption step, or perhaps brief heating would be adequate. This phase has not been explored, since it has never appeared to offer a major difficulty.

The desire for increased speed in carrying out the fluorometric method has been expressed from several quarters. In the writer's

TABLE II

SUMMARY OF COLLABORATIVE RIBOFLAVIN ASSAYS BY THE MICROBIOLOGICAL METHOD

Collaborator	Riboflavin in μg/g				Recovery of added riboflavin			
	Patent flour	Fortified flour	Fortified bread	Whole wheat flour	Flour		Bread	
					μg/g	%	μg/g	%
A	0.56	2.73	2.98	1.51	2.17	82	2.42	80
D	0.34	2.73	2.78	1.51	2.39	90	2.44	80
N	0.24	1.99	2.38	0.84	1.75	66	2.14	70
E	0.79	4.70	5.40	4.90	3.91	148	4.61	152
F	0.86	4.35	4.57	2.33	3.49	132	3.71	122
O	0.23	1.97	2.45	1.03	1.74	66	2.22	78
P	0.58	2.91	3.01	1.37	2.33	88	2.43	80
Q	0.40	3.80	4.20	1.20	3.40	128	3.80	125
Mean	0.50	3.15	3.49	1.84	2.65	100	2.99	98
Minimum	0.23	1.97	2.38	0.84				
Maximum	0.86	4.70	5.40	4.90				
The following results were obtained too late to be included in the tabulation on which the graphs were based								
S	0.40	3.10	3.40	1.33	2.70	102	3.00	99
T	0.43	1.80	1.80	1.20	1.37	52	1.37	45
U	0.44	2.27	3.05	1.20	1.73	66	2.61	86

laboratory a few experiments have considered the possibility of omitting the permanganate treatment. While this study has not been sufficiently complete to justify specific recommendations, it appears possible that in the case of enriched flour such omission can be satisfactorily made. However, for the assay of bread, the oxidation treatment must be carried out; otherwise, excessively high values will normally result.

Summary

The assay results reported by 18 collaborators for four samples of cereal products are summarized in Tables I and II. Table I consolidates the data derived by fluorometric methods and Table II the results obtained by the microbiological procedure. These data are the

basis of the several figures presented in the discussion section of this report.

Comparison of the average results obtained by microbiological and fluorometric methods shows that the former tends to given somewhat higher and more erratic results. To a considerable extent, however, this difference is due to one or two excessively high microbiological values. Based on riboflavin recoveries, both methods appear to be capable of performing fairly satisfactorily. There is, however, too wide a spread between the values from some of the laboratories. The available data do not reveal the cause of these discrepancies.

It is particularly difficult to draw definite conclusions in view of the close agreement observed by several laboratories for some of the samples and the wide variance for the others. Thus, for the microbiological method four laboratories reported practically identical values for the patent (0.40 to 0.44 μg/g) and whole wheat (1.20 to 1.33 μg/g) flours, while at the same time their values for the fortified flour and bread covered a range greater than two-fold, recoveries varying from 52 to 128% and 45 to 125%, respectively. Only one of these four laboratories recovered quantitatively the amount of added riboflavin. Why should their techniques perform apparently satisfactorily on natural products and fail on those which had been fortified?

Throughout all the collaborative results there is a marked tendency for both fortified products to exhibit the same discrepancies. Laboratories obtaining poor recoveries for the flour almost invariably obtained similar results for the bread. The same trend was observed when recoveries were excessively high. It would appear that the problems in assaying these two types of products are essentially similar.

On the basis of the above collaborative findings, it is recommended that the individual analysts give particular attention to the assay of fortified products noting their ability to "recover" added riboflavin. While this will not prove that the magnitude of their assays is correct, it will at least aid materially in guiding their assay techniques. Present procedures should not be condemned until failure to obtain good recoveries by carefully following the prescribed procedure has been adequately demonstrated. It is recommended that future collaborative activities focus their major consideration on those parts of the procedures which present greatest possibilities for error.

Acknowledgments

The author wishes to express his appreciation to the following individuals, who actively participated in this collaborative study: O. W. Barlow, Winthrop Chemical Co., Inc., Rensselaer, N. Y.; R. T. Conner, General Foods Corp., Hoboken, N. J.; Carl Nielsen, Abbott Laboratories, North Chicago, Ill.; L. W. Haas, The W. E. Long Co., Chicago, Ill.; E. B. Brown, Anheuser-Busch, Inc., St. Louis, Mo.; B. L. Oser, Food Research Laboratories, Inc., Long Island City, N. Y.; E. A. Grant, Ontario

Research Foundation, Toronto, Canada; M. W. Mead, National Grain Yeast Corp., Belleville, N. J.; Wendell Reeder, Campbell Taggart Research Corp., Kansas City, Mo.; F. E. Randall, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.; H. J. Cannon, Laboratory of Vitamin Technology, Chicago, Ill.; Betty Sullivan, Russell-Miller Milling Co., Minneapolis, Minn.; Miss Evelyn Bloom, Kraft Cheese Co., Chicago, Ill.; W. R. Urban, Omaha Grain Exchange, Omaha, Nebr.; L. K. Rochen, Lucidol Corp., Buffalo, N. Y.; G. H. Lanning, Continental Baking Co., Jamaica, N. Y.; R. T. Bohn, General Baking Co., New York City, N. Y.; H. M. Boyd, General Mills, Inc., Minneapolis, Minn.; Frank M. Strong, University of Wisconsin, Madison, Wisconsin; Esmond E. Snell, University of Texas, Austin, Texas; J. C. Bauernfeind, Hiram Walker and Sons, Inc., Peoria, Illinois.

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THE CORRELATION OF MIXOGRAMS WITH BAKING RESULTS [1]

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(Read at Annual Meeting, May 1942)

Recent developments in the determination of physical properties of flour doughs have centered on mechanisms that measure changes in the combined effects of elasticity, plasticity, and viscosity as functions of continuous mixing. The National-Swanson-Working recording dough mixer or mixograph is such a mechanism. Curves from the mechanism are called mixograms (Swanson and Johnson, 1943). Several workers have demonstrated the usefulness of recording dough mixers in studying the physical properties of doughs and have attempted to associate these properties with the baking quality of flour. While these researches have contributed knowledge of the relationship of physical properties of doughs to other quality factors, no systematic and statistical study relating mixogram characteristics directly with baking results has been reported. The study reported here was designed with that purpose in mind.

Review of Literature

American cereal chemists have contended that, to be of value, the results of physical methods of testing flour quality must be highly

[1] Contribution No. 97, Department of Milling Industry. Condensed from a thesis submitted by John A. Johnson to the graduate faculty, Kansas State College, in partial fulfillment of the requirements for the degree of Master of Science.

correlated with baking results. Geddes, Larmour, and Mangels (1934) stated that interpretation of results of physical tests for measuring flour qualities must be based upon baking results in order to be acceptable to American chemists. Malloch (1938) also contended that interpretation of recording dough mixer curves must be associated with baking results. Swanson and Working (1933) stated that results from a large number of flours from different wheat classes would be necessary to establish the relationship between curve characteristics and baking quality.

An interpretation of mixogram characteristics in the light of physical and baking properties was given by Swanson and Clark (1936). The dough development time was accepted as indicative of the amount of mixing required to develop a dough to optimum consistency. The height was found to increase directly with increasing protein content and inversely with increasing absorption. The width of the curve at the peak was thought to indicate elasticity. Swanson (1940) later modified this interpretation of curve width, because he found that a starch-water system gave a wide band. This agrees with the findings of Markley (1937). Curves exhibiting a small rate of dough weakening upon being overmixed were thought to possess the greatest tolerance to overmixing.

Swanson (1936), in a study of the relationship of curves to quality in wheat varieties, concluded that mixograms with a small area under the curve and a rapid breakdown following the peak indicate low mixing tolerance, and that curves with broad tops and amplitudes that persist beyond the peak indicate good bread flours. Because curves have characteristics that are functions of varietal differences, he suggested that preliminary testing of new varieties be made by means of the mixograph.

Larmour, Working, and Ofelt (1939) studied a series of wheat varieties at several protein levels by means of baking and curves. These authors considered that the greatest value of the curves, provided the protein content is known, is to identify the type to which a flour belongs. The curves tended to establish qualitative differences between flours that were or were not different in baking quality.

The relationship of the normal farinogram to the baking strength of western Canadian wheat samples was studied by Geddes, Aitken, and Fisher (1940). Higher simple correlation coefficients between protein content and loaf volume were obtained than between loaf volume and the principal farinogram characteristics. Partial correlation coefficients, independent of protein content, between farinogram characteristics and loaf volume were either of low order or nonsignificant. These authors concluded that no increase in precision was

obtained in estimating loaf volume from protein content by the inclusion of a farinogram measurement. The utility of the farinogram was considered to be largely as a source of accessory information such as an aid in determining correct absorption, optimum mixing time, and mixing tolerance.

It was thought that a similar study with the mixograph would be valuable. It is realized that the flour-water mixogram may not be expected to show responses of a flour to certain baking ingredients and treatment. However, it was thought valuable to study the relationship of the mixogram characteristics to baking results in such a manner as the mixogram is commonly employed, with accepted baking formulas and methods.

Materials and Methods

Sixty-three composite samples representing 12 pure wheat varieties each having 4 to 6 protein levels were experimentally milled, analyzed, and baked. Ten of the varieties (Turkey, Tenmarq, Comanche, Pawnee, Blackhull, Early Blackhull, Chiefkan, Nebred, Kanred, and Cheyenne) represent hard red winter wheat, whereas Kawvale is a semihard wheat, Clarkan a soft wheat, and both are classed as soft red winter wheats.

The wheats were milled on a Buhler experimental mill under constant atmospheric conditions of approximately 80°F and 65% relative humidity. All flour samples, prior to baking and making of mixograms, were aged for six weeks in the mill room before removal to storage at approximately 45°F.

Mixograms were made before baking, at No. 9 spring setting, with 35 g of flour (15% moisture basis). The absorptions used for the mixograms as well as those for baking (with adjustments for formulas and flour moisture) were calculated by use of a regression equation of absorption (15% moisture basis) based on protein content. The regression equation—absorption (15% moisture basis) = 0.92 flour protein + 49.03—was calculated from data obtained by determining the absorption by manual "feel" of the dough of high and low protein flours of each variety. While the flour absorption calculated from this equation was satisfactory for the samples used in this study, it may not be satisfactory for other classes of wheat or even for samples of the same class for another crop year. A similar procedure was used by Johnson and Swanson (1942). To obviate the effect that temperature fluctuations might have on the mixograms, the ingredients and apparatus were held at 77°F in the air-conditioned baking laboratory.

The flours were baked by three procedures, the malt-phosphate-bromate (MPB) formula (Aitken and Geddes, 1934), the rich formula

(Ofelt and Larmour, 1940), and the latter formula with overmixing for two minutes beyond the optimum as indicated by the mixograms. The formulas contained the following ingredients:

	MPB (grams)	Rich (grams)
Flour (15% moisture basis)	100	100
Sugar	5.0	6.0
Salt	1.0	1.5
Yeast	3.0	2.0
Dry milk solids	0.0	6.0
Shortening	0.0	3.0
$KBrO_3$	0.001	0.004
Malt syrup	0.3 (200°L)	0.25 (120°L)
$NH_4H_2PO_4$	0.1	0.0
Water	As calculated from regression equation	Plus 2% over that calculated from regression equation

The doughs for baking were mixed in a Swanson-Working type of mixer, at 96 rpm, to an optimum development previously established by the mixogram. The mixer bowl had two fixed pins in diametrically opposed positions. Preliminary studies indicated that the mixing time shown by the mixogram was about ¼ minute too long for the MPB formula and approximately ½ minute too short for the rich formula.

After mixing, the doughs were fermented at 30°C and 85% relative humidity for intervals of 105 minutes (to first punch), 50 minutes (to second punch), and 25 minutes (to pan), a total of 3 hours. The punching was done with a National pup sheeter and the molding was done with a Thompson laboratory molder. Proofing was at 30°C and 85% relative humidity for 55 minutes. The tall A.A.C.C. approved pans were used. Baking was done in a Despatch oven at 425°F for 25 minutes. All loaves were weighed and measured for volume immediately after removal from the oven. Texture, grain, and crumb color scores were made approximately 16 hours after baking. One loaf was baked from each flour on successive days until the loaf volumes checked within 25 cc.

The mixograms were segregated on basis of five main characteristics, according to the plan described by Swanson and Johnson (1942). In addition to the characteristics described by these authors, the width of the mixogram at the peak and the area under the mixogram from starting point to point of minimum mobility were measured. A simple schematic diagram of a mixogram is given in Figure 1 for convenient reference. The distance between arcs represents approximately one minute (Fig. 1). The mixing time is measured by the number of arcs or fractions thereof from the start to the point of minimum mobility, or peak. The development angle *DOT* is formed by the ascending slope and a horizontal line drawn to the left from the peak. The

range-of-tolerance angle *DOW* is formed by the ascending and descending slopes. The height *H* is determined by the number of horizontal units on the chart from the base to center of the mixogram at the peak. The width M–R is the distance between two lines drawn

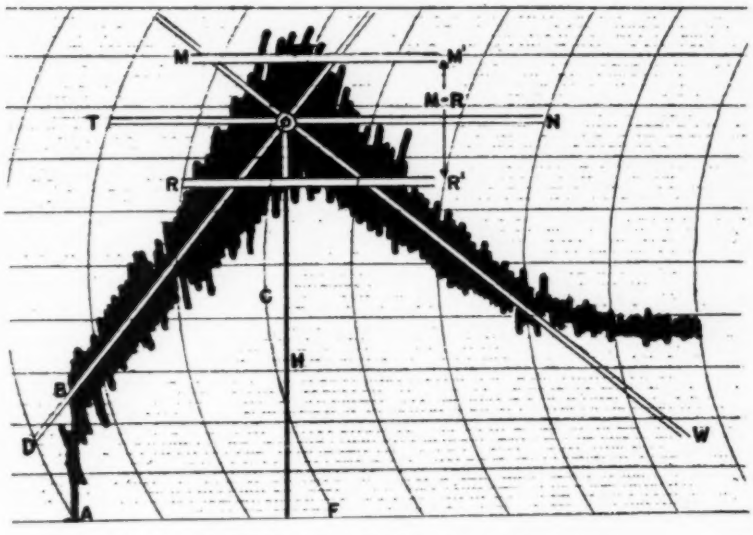

Fig. 1. Schematic diagram showing mixogram measurements.

at the top and bottom of mixogram band at the peak and is expressed in horizontal chart units. The weakening angle *WON* is formed by the first portion of the descending slope and a horizontal line drawn from the right of the peak. The area is the portion enclosed beneath the mixogram during the dough development period and is measured with a planimeter. It is enclosed by the boundary *ABOCF*.

Experimental Data

The loaf volumes obtained from both formulas, the protein contents, and mixogram measurements are presented in Table I. The data are arranged in order of increasing protein content within each variety. The mixograms from which the measurements were taken are presented in Figure 2 and are arranged from left to right in order of increasing protein content for each variety.

Grain, texture, and crumb color scores are not included in Table I, because they were subjective and not suitable for statistical treatment. A brief summary of these values is given, however. With increasing protein content the texture of the bread was found to improve, while the grain became increasingly open, reaching an optimum at about 12%

TABLE I

Protein Content, Loaf Volume, and Mixogram Data for Flours

Serial number	Curve No., Fig. 1	Flour protein [1]	Loaf volume			Mixogram characteristics						
			MPB, optimum mix	Rich, optimum mix	Rich, decrease by overmix [2]	Mixing time	Angle *DOT*	Area	Angle *DOW*	Height *H*	Width R–M	Angle *WON*
		%	*cc*	*cc*	*cc*	*min*	*deg*	*cm²*	*deg*	*units*	*units*	*deg*
TURKEY												
1688	1	8.1	600	700	37	3.7	15	18.6	164	36	0.9	4
1689	2	9.0	618	745	37	3.4	18	21.2	156	40	1.2	7
1690	3	10.4	770	843	75	2.8	59	19.9	123	52	1.3	18
1691	4	11.6	828	903	83	2.5	58	15.4	102	58	1.6	20
1692	5	13.2	938	1025	147	2.7	54	19.3	100	68	2.0	26
1693	6	14.5	1055	1085	127	3.0	50	26.3	93	76	2.5	35
TENMARQ												
1694	7	8.6	603	655	30	6.0	6	27.6	167	30	0.9	6
1695	8	9.3	665	700	32	3.7	18	23.1	157	40	1.3	6
1696	9	10.5	773	780	47	3.1	31	23.1	135	52	1.7	14
1697	10	11.8	855	865	80	3.3	35	27.6	123	58	1.8	20
1698	11	13.2	911	970	127	3.0	50	27.0	109	68	2.0	20
1699	12	15.2	1050	1066	126	3.5	45	30.2	103	76	2.4	32
COMANCHE												
1737	13	9.2	640	712	47	4.9	14	32.8	158	43	1.1	8
1738	14	10.4	725	752	29	3.8	31	29.6	134	58	1.6	15
1739	15	11.9	829	873	98	3.8	35	30.8	130	56	1.6	15
1740	16	13.3	909	950	132	2.8	49	25.1	108	70	2.3	23
1741	17	14.5	1007	993	128	2.6	54	24.4	96	76	2.0	29
1742	18	16.1	1238	1188	143	3.6	45	24.7	108	76	2.7	29
PAWNEE												
1731	19	8.6	585	697	37	2.5	20	12.9	161	33	0.9	4
1732	20	9.5	655	708	58	2.3	40	13.5	127	45	1.2	10
1733	21	10.3	677	740	45	2.0	58	12.2	104	54	1.5	18
1734	22	11.8	850	868	100	1.8	53	15.4	104	60	1.6	22
1735	23	13.2	903	900	117	1.6	62	14.1	89	68	2.0	29
1736	24	14.9	1080	1028	145	2.1	66	17.4	74	80	2.2	38
BLACKHULL												
1700	25	8.7	625	725	25	3.5	20	18.6	153	36	0.8	7
1701	26	9.4	678	735	37	2.3	42	13.5	120	46	1.0	18
1702	27	10.7	740	852	47	2.3	49	14.1	110	53	1.1	21
1703	28	12.3	853	920	132	2.0	59	16.1	90	63	1.2	30
1704	29	13.6	909	966	—	2.0	61	15.4	90	64	1.7	29
1705	30	14.3	980	1045	142	2.0	60	16.1	89	70	1.9	32

[1] Moisture basis 15%.
[2] Rich formula optimum mix loaf volume minus rich formula loaf volume with 2-minute overmixing.

TABLE I—(*Continued*)

Serial number	Curve No., Fig. 1	Flour protein [1]	Loaf volume			Mixogram characteristics						
			MPB, optimum mix	Rich, optimum mix	Rich, decrease by overmix [2]	Mixing time	Angle *DOT*	Area	Angle *DOW*	Height *H*	Width R–M	Angle *WON*
		%	*cc*	*cc*	*cc*	*min*	*deg*	*cm²*	*deg*	*units*	*units*	*deg*
EARLY BLACKHULL												
1725	31	9.4	615	748	23	2.3	44	13.5	119	46	1.3	16
1726	32	10.7	709	783	33	1.8	64	11.6	86	60	1.8	30
1727	33	12.0	783	840	25	2.0	58	14.8	95	60	1.6	27
1728	34	13.3	904	940	105	2.0	60	13.5	92	65	1.8	28
1729	35	15.2	982	1038	153	2.3	58	18.6	88	71	1.8	34
1730	36	16.3	1070	1135	205	1.9	66	16.7	68	81	2.3	46
CHIEFKAN												
1710	37	9.9	645	695	97	2.0	65	10.3	95	56	1.4	19
1711	38	10.9	707	705	100	1.8	70	14.8	82	65	1.4	28
1712	39	12.1	745	740	102	1.8	75	10.3	70	67	1.6	35
1713	40	12.9	808	813	106	1.8	72	14.1	70	76	1.8	38
1714	41	15.0	853	880	142	2.0	70	14.1	71	78	2.5	40
NEBRED												
1706	42	10.9	790	895	107	2.5	45	20.6	116	62	1.7	17
1707	43	12.0	825	899	79	2.6	49	25.1	113	70	1.8	21
1708	44	13.2	875	965	122	2.8	45	24.4	115	67	2.1	20
1709	45	15.0	1035	1078	98	2.8	51	28.9	95	84	3.2	34
KANRED												
1743	46	10.8	692	733	28	2.4	45	19.3	121	58	1.6	13
1744	47	11.8	783	793	70	2.0	60	14.8	97	63	1.7	21
1745	48	13.0	907	875	82	1.8	70	12.2	88	70	2.0	26
1746	49	14.7	1010	965	155	1.8	67	10.9	83	73	2.0	30
CHEYENNE												
1747	50	9.3	627	700	67	3.0	33	19.9	134	50	1.1	11
1748	51	9.9	675	725	87	3.5	30	23.8	140	50	1.5	11
1749	52	11.2	698	740	87	3.5	39	28.9	124	64	1.5	16
1750	53	12.8	825	855	157	2.9	50	21.2	110	66	1.7	20
KAWVALE												
1715	54	7.8	593	628	23	3.0	11	16.7	160	34	0.9	8
1716	55	9.3	650	703	65	2.5	37	15.4	130	48	1.2	12
1717	56	10.3	709	748	63	2.3	50	14.8	112	53	1.4	18
1718	57	12.3	845	889	72	2.2	50	16.1	111	54	1.4	19
1719	58	13.0	933	935	75	2.5	50	18.6	100	62	1.4	29
1720	59	14.3	1008	1023	103	2.8	47	23.1	105	64	1.6	25
CLARKAN												
1721	60	9.3	668	758	103	2.0	11	11.6	160	36	0.8	7
1722	61	10.3	703	765	58	1.8	32	10.3	135	41	1.0	13
1723	62	11.5	730	840	120	1.4	47	10.3	114	48	1.1	18
1724	63	12.9	770	853	48	1.5	60	11.6	82	64	1.6	39

to 13% protein levels. The bread from the rich formula, in general, had better texture and grain than that from the MPB formula. Bread from Tenmarq, Turkey, Comanche, Kawvale, Pawnee, Nebred, and Blackhull had slightly better texture and grain than that from the other varieties. The grain of the Chiefkan, Cheyenne, and Early Blackhull breads was either open or had heavy cell walls.

The crumb color scores indicated that Turkey, Kawvale, and Nebred samples were decidedly yellow, indicating high pigmentation. Turkey and Nebred samples had a higher luster of crumb than Kawvale. Tenmarq samples exceeded all other samples in creamy whiteness of crumb color with a highly desirable sheen. Clarkan samples had a clear white but dull crumb color.

Discussion

Loaf volume has been used as the chief criterion of baking quality in this study, because it is the only objective value obtained by actual measurement. Sufficient evidence is found in the literature to suggest that loaf volume is a measurement of flour quality provided adequate baking methods are used (Aitken and Geddes, 1939).

Covariance analysis of loaf volume data: An examination of the values for loaf volume in Table I show that the two formulas did not give the same ranking in loaf volume. To test the significance of this apparent disagreement between the two formulas, the loaf volume and protein data were submitted to covariance analysis. The covariance analysis data for both formulas combined are presented in Table II. From this

TABLE II

ANALYSIS OF COVARIANCE OF LOAF VOLUME AND PROTEIN CONTENT DATA FOR THE MPB AND RICH FORMULAS

Source of variation	Degrees of freedom	Mean square
Difference for testing adjusted variety means	11	14,989.4†
Difference for testing between formulas	1	69,900.0†
Difference for testing variety-formula interaction	11	4,478.7†
Residual error	101	1,038.4

† Highly significant.

table it becomes evident that a significant difference existed between varieties and between formulas. A significant variety-formula interaction indicated that varieties reacted differently to treatment accorded them by the baking formula. Because of this interaction, a study of the relationship of mixogram characteristics to baking results must necessarily be made separately for each baking formula.

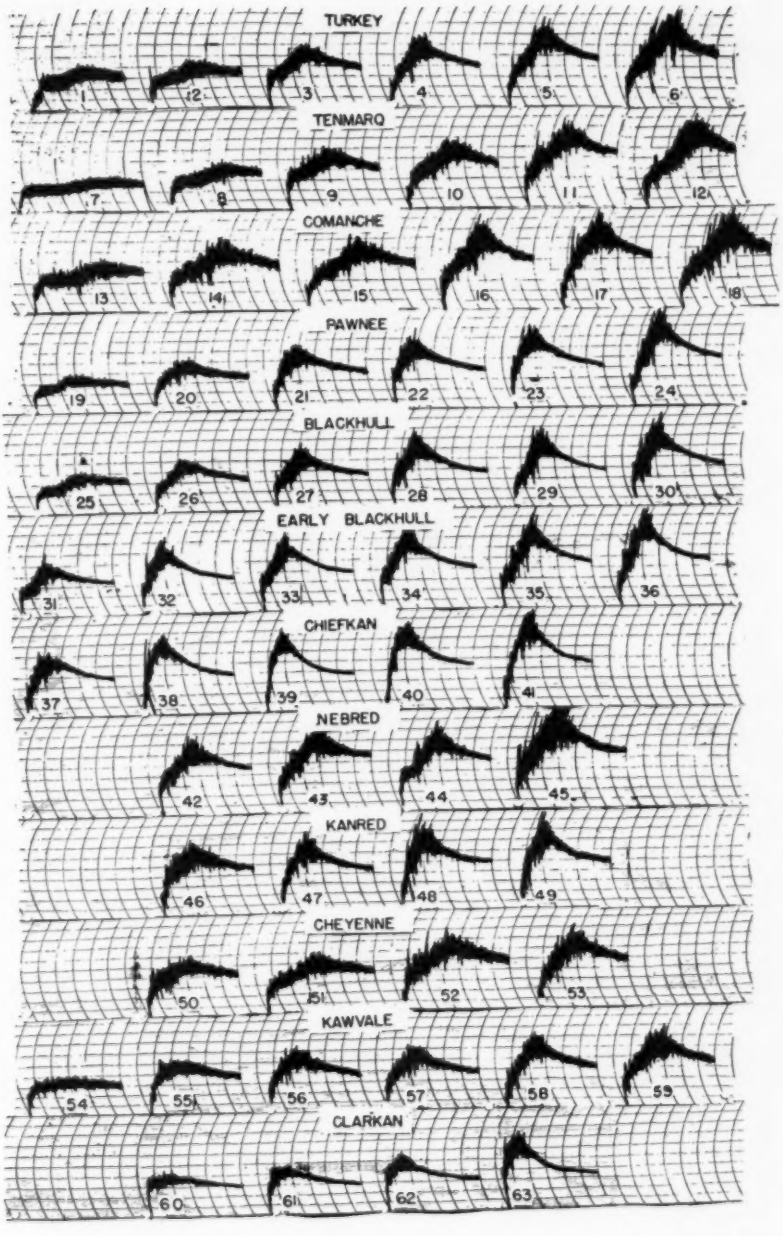

Fig. 2. Mixograms of flours arranged in order of increasing protein content from left to right for each variety. Numbers correspond to those given in Table I.

Covariance analysis of the loaf volume data, with each formula treated separately, are presented in Table III. The mean adjusted loaf volumes proved significantly different for the different varieties, regardless of formula. The rich formula gave greater differentiation between samples, as is shown by the *F* ratio 5.47 for the MPB formula compared to the *F* ratio 15.81 for the rich formula.

TABLE III

ANALYSIS OF COVARIANCE OF THE LOAF-VOLUME AND PROTEIN-CONTENT DATA, TREATING EACH FORMULA SEPARATELY

Source of variation	Degrees of freedom	Mean square
Difference for testing adjusted mean loaf volume for MPB formula	10	7,392.0†
Residual error	50	1,352.1
Difference between individual variety regressions of loaf volume on protein content	39	742.7
Differences for testing adjusted mean loaf volume for the rich formula	10	12,573.1†
Residual error	50	795.4
Differences between individual variety regressions of loaf volume on protein content	39	567.11

† Highly significant.

Relationship of loaf volume to protein content as reflected by varieties: The significance of the increase in loaf volume with increments in protein for individual varieties was tested and the results are also presented in Table III. It appears that the samples as a group could be considered representative of the same population as far as the regression coefficient of loaf volume on protein content is concerned. Thus, these data do not appear to support the view that regression of loaf volume on protein content is a varietal characteristic. If many more samples for each variety had been available, it is possible that significant differences of regression of loaf volume on protein content might then have been found.

The relationship between loaf volume and protein content is shown graphically in Sections A and B of Figure 3. It was evident with either formula that a close linear relationship existed. Correlation coefficients for this study are summarized in Table IV. In this table the total simple correlation coefficients between loaf volume and protein content for the MPB formula (+ .9417) and for the rich formula (+ .9214) are shown. Variation in protein content thus accounts for 88% and 84% of the total variation in loaf volume for each formula respectively. The 12% and 16% of the total variation in loaf volume

not accounted for by the protein content, for each formula respectively, can partly be attributed to different qualities of the protein among the varieties and partly to random errors of the baking procedures.

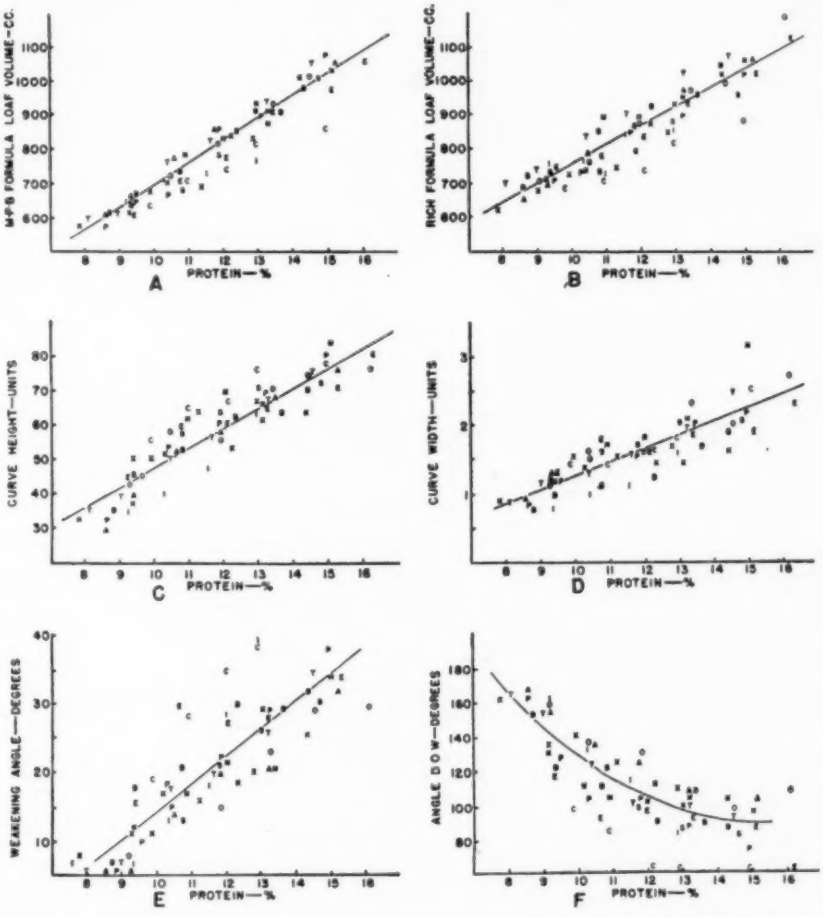

Fig. 3. Relationship between protein content and loaf volume, mixogram height, width, weakening angle, and angle *DOW*.

The symbols represent the varieties as follows:

Turkey	T	Chiefkan	C
Tenmarq	A	Nebred	N
Comanche	O	Kanred	S
Pawnee	P	Cheyenne	X
Blackhull	B	Kawvale	K
Early Blackhull	E	Clarkan	I

Relationship between protein content and certain mixogram characteristics: The relationships found between mixogram height, width,

weakening angle, and protein content as shown in Sections C, D, and E of Figure 3, were linear and the degrees of association were high. The simple correlation coefficient (Table IV) between height and protein content was + .9189, that for width was + .8533, while that for the weakening angle was + .8362.

TABLE IV

SUMMARY OF SIMPLE, PARTIAL, AND MULTIPLE CORRELATION COEFFICIENTS FOR LOAF VOLUME, PROTEIN, AND MIXOGRAM CHARACTERISTICS

($N = 63$)

Variables correlated		Correlation coefficients [1]		
X	Y	Simple r_{xy}	Partial [2] $r_{xy \cdot p}$	Multiple [3] R
Protein content, %	MPB loaf volume, cc	+.9479		
Protein content, %	Rich loaf volume, cc	+.9214		
Protein content, %	Curve height, units	+.9189		
Curve height, units	MPB loaf volume, cc	+.8455	−.2029	
Curve height, units	Rich loaf volume, cc	+.7906	−.3558	+.9323
Protein content, %	Curve width, units	+.8533		
Curve width, units	MPB loaf volume, cc	+.8295	+.1244	
Curve width, units	Rich loaf volume, cc	+.7853	+.0046	
Protein content, %	Angle *WON*, degree	+.8362		
Angle *WON*, degrees	MPB loaf volume, cc	+.7202	−.4146	+.9595
Angle *WON*, degrees	Rich loaf volume, cc	+.6971	−.3443	+.9311
Mixing time, min.	Angle *DOT*, degree	−.6815		
Mixing time, min.	Area, cm^2	+.6958		
Area, cm^2	MPB loaf volume, cc	+.2228		
Area, cm^2	Rich loaf volume, cc	+.2204		
Protein content, %	Decrease in loaf volume, cc	+.6901		
Angle *WON*, degrees	Decrease in loaf volume, cc	+.6365	+.4736	+.6986

[1] Five percent level of significance, $r = +.250$ (Snedecor, 1940).
[2] Protein content held constant.
[3] Includes variable Y and protein content with variable X.

The relationship existing between angle *DOW* (believed to indicate range of mixing tolerance) and protein content is shown in Figure 3F. It appeared that the relationship between angle *DOW* and protein content was curvilinear and negatively correlated. Since protein content has a pronounced influence on the sharpness of the peak (angle *DOW*) it is well to know the protein contents of samples when comparing mixogram patterns.

Relationship between loaf volume and certain mixogram characteristics: Linear relationships between loaf volume for both formula and mixogram height, width and weakening angle are shown in Figure 4.

The degree of association between mixogram height and loaf volume ($r = +.8455$ for the MPB formula and $r = +.7906$ for the rich formula) was not as high as the degree of association between loaf volume and protein content ($r = +.9479$ for the MPB and $r = +.9214$

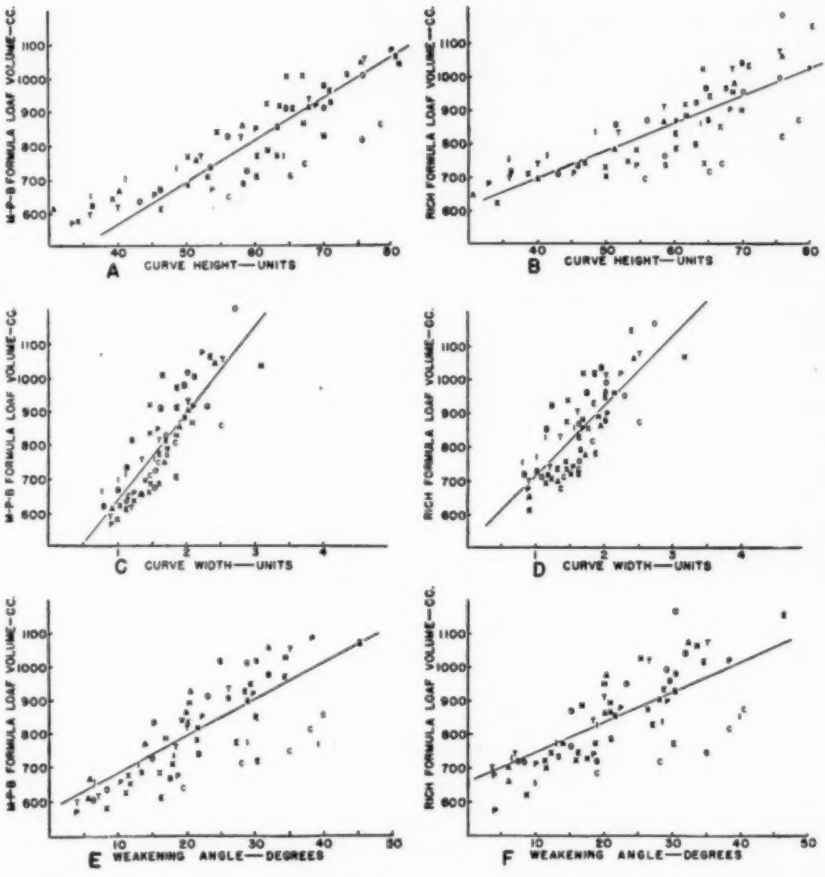

Fig. 4. Relationship between loaf volume and mixogram height, width, and weakening angle. (See Fig. 3 for legend.)

for the rich formula) (Table IV). These differences were tested and found to be significant. Thus it is apparent that height of the mixogram would not furnish as good an estimate of loaf volume as would protein content. The partial correlation coefficients independent of protein content were calculated to permit a study of the relationship of mixogram height to protein quality factors (Table IV). The negative correlation coefficient, $-.2029$, between the MPB-formula loaf volume and mixogram height, independent of protein content, was

nonsignificant. This indicated that when compared on a common protein level, no relationship existed between mixogram height and loaf volume. A similar partial correlation coefficient, − .3558, for the rich formula, was significant but of low order. The multiple correlation coefficient, + .9323, rich formula (loaf volume times protein content, including mixogram height), was probably not significantly different from the simple correlation coefficient, + .9214, between protein content and loaf volume. Thus little would be added to the accuracy with which loaf volume could be estimated from protein content by inclusion of the mixogram height.

Mixogram width, *M–R*, was not as highly correlated with loaf volumes produced by either formula as was mixogram height or protein content. When we compare the correlation coefficients between width *M–R* and loaf volume, + .8295 for the MPB formula and + .7853 for the rich formula, with + .9479, and + .9214 between loaf volume and protein content for each formula respectively, it is apparent that curve width did not furnish as good an estimate of loaf volume as did protein content. The partial correlation coefficients, independent of protein content, between loaf volume and mixogram were nonsignificant, thus indicating that width of the mixogram was not associated with protein quality factors as reflected in loaf volume. Loaf volume was associated with mixogram width only because of the high degree of association between protein content and loaf volume on the one hand and protein content and curve width on the other.

The weakening angle, *WON*, was not as highly correlated with loaf volume as was protein content, mixogram height, or width. The simple correlation coefficients between loaf volume and weakening angle were moderately high (+ .7202 for the MPB formula and + .6971 for the rich formula). The partial correlation coefficients, independent of protein content, between weakening angle and loaf volume from either formula were negative and significant. However, the magnitude of these partial correlations, − .4146 for the MPB formula and − .3443 for the rich formula, suggested that if varieties are compared on a uniform protein content basis, the weakening angle will not give any marked degree of accuracy in predicting the baking performance of the flour.

The multiple correlation coefficient + .9595 (MPB loaf volume × protein content, including weakening angle) was probably not significantly different from the simple correlation coefficient + .9479, between the same loaf volume and protein content. It is thus likely that little information would be added to improve the estimate of loaf volume from protein content by inclusion of the weakening angle. The same conclusion may be made for the rich-formula loaf volumes

by comparing the multiple correlation coefficient, + .9311, with the simple correlation coefficient, + .9214.

Relationship of mixing time to protein content: Several workers have studied the relationship between mixing time of flour and other quality measures. Many of these studies have included the measurement of

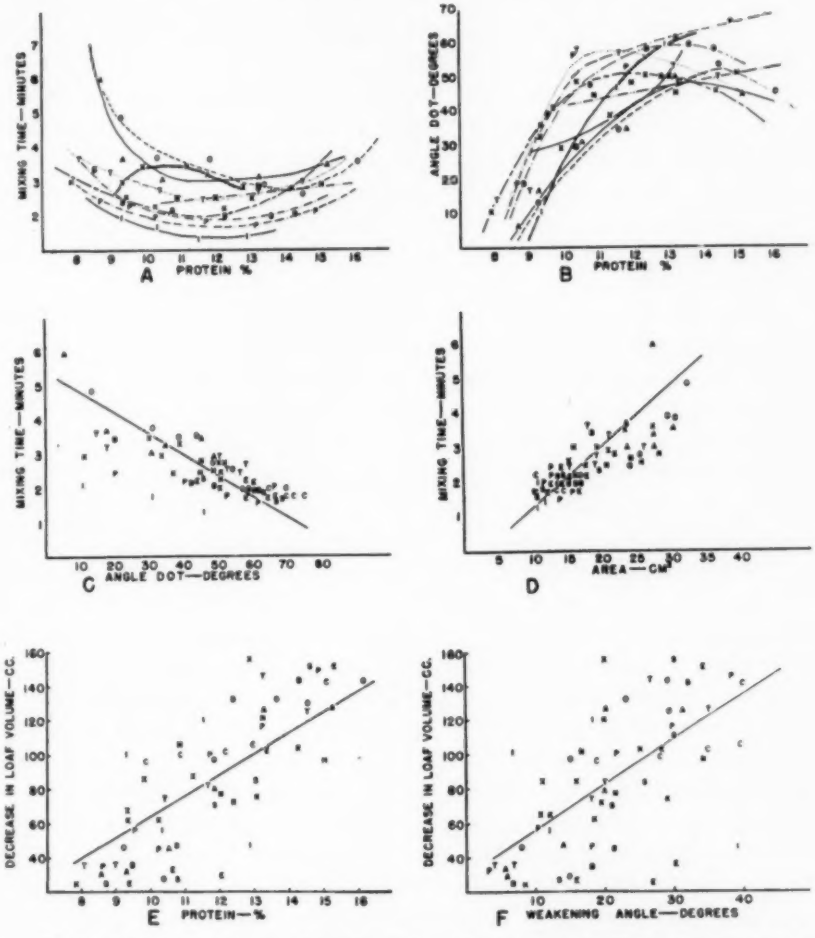

Fig. 5. Relationship between mixing time and protein content, angle *DOT*, and area under curve to point of minimum mobility; between protein content, angle *DOT*, and decrease in loaf volume; and between decrease in loaf volume and weakening angle. (See Fig. 3 for legend.)

the mixing time either with the farinograph or mixograph. It should be noted that the data from the farinograph, which has a gentle kneading action on the dough, do not agree with those obtained from the mixograph, which has a rigorous mixing action. Markley (1938), Aitken and Geddes (1939), and Markley, Bailey, and Harrington (1939)

have shown that the amount of mixing required to develop dough to minimum mobility with the farinograph increased with increments of protein. The opposite has been found for the mixograph by Bayfield, Working, and Harris (1941) and Ofelt and Sandstedt (1941).

The relationship between mixing time and protein content found in this study is shown in Figure 5A. The mixing time decreased with increments of protein up to about 12% and then tended to increase again as the protein content became greater, except for Nebred and Cheyenne. No explanation is offered for the failure of these two to follow the general trend of the other varieties. The lack of agreement in the relationship between mixing time and protein content from farinogram and mixogram data may be due to the nature of the mixing action of the two machines. The farinograph has a gentle kneading action, while the mixograph, like high-speed commercial mixers, has a pull-fold-repull type of action.

The mixing time as determined from the mixogram, with adjustments for formula, was found to parallel the mixing time required to mix doughs to optimum development for baking. This development was associated with a dry "feel" and smoothness of the dough. This was in agreement with the conclusions of Bohn and Bailey (1937).

Relationship between development angle, protein content, and mixing time: The relationship between development angle *DOT* and protein content is shown in Figure 5B and that between development angle and mixing time is shown in Figure 5C. The curvilinear relationship between development angle *DOT* and protein content, and also between mixing time and protein content, suggests some degree of association between angle *DOT* and mixing time. The relationship between angle *DOT* and mixing time appears linear. This association is inverse, since the correlation coefficient between development angle *DOT* and mixing time was − .6815 (Table IV).

Energy relationships: Dough mixing time has been considered to be indicative of the amount of energy input required to mix a dough to optimum development. That mixing time indicates to some extent the energy input is shown in Figure 5D, and also by the simple correlation coefficient between mixing time and area under mixogram to minimum mobility, which was + .6958 (Table IV). The area under the mixogram to minimum mobility is not an exact measurement of energy input since it also includes friction, but since friction is fairly constant, area is a mixogram characteristic which best describes energy input. The area may be considered as a measurement of work done, because the height of the mixogram indicates the force required to move the mixing pins through the dough, whereas mixing time indicates the time during which the force operates. Area included in the mixo-

gram to point of minimum mobility was not found significantly correlated with loaf volume with either baking formula. The nonsignificant correlation coefficients were + .2228 and + .2204 (Table IV).

The relationship between sensitivity to overmixing and protein content and weakening angle WON: The sensitivity to overmixing is shown by the magnitude of the weakening angle *WON*. It has been found that varieties with a mixogram having a rapid breakdown are undesirable because of their sensitivity to overmixing and therefore are more difficult to handle in the bake shop.

The relationship between protein content and the decrease in loaf volume caused by overmixing is shown in Figure 5E. The amount of decrease in loaf volume increased in a linear relationship to the increase in protein content. The simple correlation coefficient, + .6901 (Table IV) between protein content and decrease in loaf volume indicated that high protein doughs are subject to greater damage from overmixing, corroborating the conclusion of Markley and Bailey (1939).

The relationship between decrease in loaf volume and weakening angle *WON* approached linearity (Fig. 5F). The correlation coefficient + .6365, between weakening angle and the decrease in loaf volume was probably not significantly different from the coefficient, + .6901, between protein content and decrease in loaf volume. The partial correlation coefficient, + .4736, independent of protein content, between weakening angle and decrease in loaf volume suggested that varieties which exhibit larger weakening angles are subject to greater damage by overmixing. That the varieties Chiefkan and Early Blackhull are sensitive to overmixing is shown by their mixograms in Figure 1. The multiple correlation coefficient, + .6986 (decrease in loaf volume × protein content, including weakening angle) was probably not significantly different from the simple correlation coefficient, + .6901, between decrease in loaf volume and protein content.

From observation of the weakening angles shown in Figure 1 and by assuming that the angle *WON* expressed sensitivity to overmixing, it was anticipated that a higher correlation than actually resulted would be obtained between weakening angles and decrease in loaf volumes. It is probable that overmixing produces several effects in dough. That overmixing does change the physical properties of doughs was indicated by increase in the weakening angle *WON* of the mixogram. It may be that the protein network was "broken down" more or less by mechanical treatment. This, however, was not always reflected in the final bread, at least not to the extent indicated by the weakening angles. That mixing in air causes certain effects similar to those caused by oxidation has been shown by Baker and Mize (1937). It may be that overmixing causes overoxidation. That some doughs

have greater tendency to recover the normal physical state after severe mixing than others was shown by Munz and Brabender (1940). It may be that both these factors tend to lower the correlation between weakening angles and the damage to bread by overmixing.

Summary

Twelve pure wheat varieties, each with four to six protein levels, were milled, analyzed, and baked by three procedures. Mixograms were made and segregated on the basis of important characteristics and the relationships studied statistically.

Statistical treatment of combined loaf-volume data of the MPB and rich formulas showed significant variety-formula interaction. Covariance analysis of the loaf-volume data within a formula showed that mean loaf volumes of varieties, after adjustment for protein content, were significantly different. When all varieties were considered as a group, then individual variety regressions of loaf volume on protein content were not significantly different. The rich formula gave greater differentiation between samples than the MPB formula.

Height, width, and weakening angle of the mixogram were positively correlated with protein content and loaf volume of either formula. Loaf volumes were more closely associated with the protein content than with any of the mixogram characteristics. The height, independent of protein content, was not significantly correlated with loaf volume obtained with the MPB formula, while the rich formula gave a negative significant correlation. Width, independent of protein content, was not found significantly correlated with loaf volume by either formula. The weakening angle, independent of protein content, was negatively correlated with loaf volume by either formula. The multiple correlation coefficients, including protein content and any one of the three mixogram characteristics, being correlated with loaf volume from either formula, were probably not significantly different from the simple correlation coefficients between loaf volume and protein content. Mixogram characteristics tended to reflect baking strength of a flour mainly because of the high correlation between loaf volume and protein content, on the one hand, and between protein content and the mixogram characteristic on the other. The angle expressing range of mixing tolerance tended to decrease with increasing protein but not in a linear fashion.

The relationship of mixing time and of development angle *DOT* with protein content was curvilinear. Development angle and mixing time were moderately and negatively correlated, the relationship being linear. Mixing time was only moderately correlated with areas under mixogram from starting point to point of minimum mobility (peak).

Area was presented as a measurement of energy input required to mix a dough to optimum development. Area was not significantly correlated with loaf volume obtained by either the MPB or rich formula.

Overmixing the doughs resulted in a decrease in loaf volume, the largest being in the high-protein samples. A fair degree of association between decrease in loaf volume and the weakening angle *WON* was found. The weakening angle, independent of protein content, was positively correlated with decrease in loaf volume caused by overmixing dough.

Conclusions

The most important use of the mixograph appears to be that of furnishing information that supplements baking data. Thus the mixogram gives information regarding mixing requirements, mixing tolerance, and varietal pattern. The varietal pattern tends to establish qualitative differences between flours that may or may not have different baking qualities. In this way flours of known inferior baking qualities may be readily distinguished and discarded as unsuitable for bread making.

Acknowledgments

Acknowledgments are due Dr. H. C. Fryer, Department of Mathematics, for valuable assistance with the statistical treatment of the data, and to Professor A. L. Clapp, Department of Agronomy, for the wheat samples used in this study.

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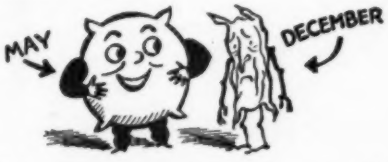

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